IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Patent Application of:	Confirmation No.: 4666
Orville G. KOLTERMAN *et al.*	Art Unit: 1646
Application No.: 09/756,690	Examiner: Dong Jiang
Filed: January 9, 2001	Atty. Docket No.: 249/124US / 18528.451

Title: Use of Exendins and Agonists Thereof for Modulation of Triglyceride Levels and Treatment of Dislipidemia

APPEAL BRIEF UNDER 37 C.F.R. § 41.37

Mail Stop Appeal Brief – Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This is an appeal from the rejection of the claims pending in the above-captioned patent application. A Notice of Appeal was filed on March 13, 2007. Authorization to charge the official fees for this filing is given in the accompanying transmittal letter.

1. Real Party in Interest

The real party in interest is Amylin Pharmaceuticals, Inc., a Delaware corporation with offices at 9360 Towne Centre Drive, Suite 110, San Diego, CA 92121

2. Related Appeals and Interferences

Appellants are unaware of any related appeal or interference that may have a bearing on the Board's decision in the present Appeal.

3. Status of Claims

Claims 1-15, 24-37 and 41 are pending. Claims 16-23 and 38-40 are canceled. Claims 1-15, 24-37 and 41 stand finally rejected under 35 U.S.C. § 103. Appellants appeal the rejections of all pending claims.

4. Status of Amendments

Appellants have not filed any amendments subsequent to the Office Action mailed December 14, 2006. However, Appellants representatives, Dianne Rees and James Butler, participated in a telephonic interview on March 1, 2007 with Examiners Diong Jiang and Gary Nickol, during which the cited prior art and obviousness rejections were discussed. However, an agreement was not reached, and the present Appeal was subsequently filed.

5. Summary of Claimed Subject Matter

The claimed subject matter includes that directed to methods of lowering triglyceride in a subject in need thereof by identifying the subject and administering to the subject a therapeutically effective amount of an exendin or exendin agonist (see, *e.g.*, para. [0023]-[0025])

Independent Claim 1: The claimed subject matter includes methods for lowering triglyceride levels in a subject in need thereof, comprising:

identifying a subject having elevated postprandial triglyceride levels (see, *e.g.*, para. [0023], [0029]; and

administering to said subject a therapeutically effective amount of an exendin or an exendin agonist, wherein said subject's postprandial triglyceride levels are lowered (see, *e.g.*, para. [0023]-[0025]).

Independent Claim 10: The claimed subject matter includes methods for lowering plasma triglyceride concentrations in a subject with heart disease, comprising:

identifying a patient with heart disease having elevated postprandial triglyceride levels (see, *e.g.*, para. [0023], [0029]); and

administering to said subject a therapeutically effective amount of an exendin or an exendin agonist, wherein said subject's postprandial triglyceride levels are lowered (see, *e.g.*, para. [0023]-[0025]).

Independent Claim 24: The claimed subject matter includes methods for lowering postprandial triglyceride levels in a subject in need thereof, comprising:

identifying a patient having elevated postprandial triglyceride levels (see, *e.g.*, para. [0023], [0029]); and

administering to said subject a therapeutically effective amount of an exendin or an exendin agonist, wherein said subject's postprandial triglyceride levels are lowered (see, *e.g.*, para. [0023]-[0025]).

A copy of the claims on appeal is attached hereto in the Claims Appendix.

6. Grounds of Rejection to be Reviewed on Appeal

The grounds of rejection to be reviewed are:

(a) whether claims 1-14, 24-36 and 41 are unpatentable under 35 U.S.C. § 103 over Karpe *et al.* (Metabolism, 1999, 48:301-307) and Beeley *et al.* (WO 98/30231);

(b) whether claims 15 and 37 are unpatentable under 35 U.S.C. § 103 over Karpe *et al.* and Beeley *et al.*, in view of Wagle *et al.* (US Patent 6,326,396).

7. Argument

A. Rejection under 35 U.S.C. § 103 over Karpe *et al.* and Beeley *et al.*

The Office has failed to establish that one of ordinary skill in the art would have found it obvious to combine the teachings of Karpe *et al.* and Beeley *et al.*. Karpe *et al.* discloses that postprandial elevation of plasma triglycerides is more closely linked to coronary heart disease (CHD) than the fasting level (page 301, second paragraph of left column). Beeley *et al.* discloses the use of exendins and exendin agonists to inhibit food intake (page 7, lines 23-25; Examples 1-4, pages 35-39). According to the Office, Beeley *et al.* also discloses the use of exendins and exendin agonists to reduce plasma lipid levels and cardiac risk. *See* Office Action mailed December 14, 2006, page 4, lines 9-12. Beely *et al*, however, contains no teaching re-

garding the use of exendins to lower triglycerides. In fact, Beeley *et al*, does not mention triglycerides at all. As explained in detail below, since it is known in the art that the lowering of lipids in general does not predict change in a particular lipid component, one of ordinary skill in the art would neither have expected exendins and exendin agonists to lower plasma triglyceride levels, nor have found it obvious to combine Beeley *et al.*'s exendins and exendin agonists with Karpe *et al.*'s subjects having elevated triglyceride levels.

Even though Beeley *et al.* does not disclose lowering plasma triglyceride levels or identifying subjects with elevated triglyceride levels, the Office appears to argue that subjects at cardiac risk inherently possess elevated triglyceride levels, and that methods of inhibiting food intake inherently lower plasma triglycerides. Office Action mailed December 14, 2006, page 4, lines 18-28. However, that which is inherent, but unrecognized in the prior art, cannot form a proper basis for rejecting the claimed subject matter as obvious under § 103. *See In re Shetty*, 566 F.2d 81, 86 (CCPA 1977). Obviousness cannot be predicated on what is not known at the time an invention is made, even if the inherency of a certain feature is later established. *See In re Rijckaert*, 9 F.2d 1531 (Fed. Cir. 1993). Hence, the Office improperly relied upon the alleged inherency of claim limitations in its construction the rejection under § 103.

Even assuming, *arguendo*, that the Office's reliance upon the alleged inherency of claim limitations is proper, the ground of rejection flounders on the Office's failure to establish inherency. Inherency requires a certainty that a property or characteristic exists. "Inherency, however, may not be established by probabilities or possibilities." *Continental Can Co. v. Monsanto Co.*, 948 F.2d 1264, 1269 (Fed. Cir. 1991) (internal quotations omitted). Yet the Office fails to show that methods of inhibiting food intake as taught by Beeley *et al.* necessarily lower plasma triglyceride levels. In fact, evidence suggests that reducing food intake does not necessarily result in lowering triglyceride levels. For example, one review article indicates that a reduction in food intake left triglyceride levels unchanged but lowered other plasma lipid levels. Bravata *et al.*, *JAMA*, Vol. 89, No. 4, pp. 1837-1850 (April 9, 2003) (Exhibit 1 in Evidence Appendix).

In an effort to identify a link between reducing food intake and lowering triglyceride levels, the Office cites Beeley *et al.*'s remarks on the uses of exendins or exendin agonists in

reducing plasma lipid levels and cardiac risk. *See* Office Action mailed December 14, 2006, page 4, lines 9-12. However, as discussed herein, the Office has failed to show that a reduction of plasma lipid levels generally or cardiac risk necessarily results in the lowering of triglyceride levels specifically as is required to support a rejection based on inherency.

Triglycerides represent one of several classes of plasma lipids. Others include cholesterol-containing lipids such as high-density lipoproteins (HDL) and low-density lipoproteins (LDL). These classes of plasma lipids are differentially regulated. As a result, a number of therapeutic agents affect one class of plasma lipids without affecting another. For example, a combination of enalapril and valsartan reduced total cholesterol and LDL levels, increased HDL levels, and had no effect on triglyceride levels in patients with diabetes and hypertension. *See* Gaudio *et al.*, *J. Cardiovas. Pharmacol*, Vol. 45, No. 4, pp. 362-366 (April 2005) (Exhibit 2 in Evidence Appendix). In another study, hormonal replacement resulted in significant lowering of total cholesterol, HDL and LDL levels, without any significant changes in triglyceride levels. *See* Ödmark *et al.*, *Maturitas*, Vol. 48, pp. 137-146 (2004) (Exhibit 3 in Evidence Exhibit). Even in patients with hyperlipidemia, lipid-lowering drugs differentially affect lipid profiles. *See* Saklamaz *et al.*, *Metabolism Clinical and Experimental*, Vol. 54, pp. 677-681 (2005) (Exhibit 4 in Evidence Exhibit, discussing the failure of pravastatin, a HMG-CoA reductase inhibitor, to lower plasma triglyceride levels while lowering LDL levels). Yet another publication teaches that a class of compounds known as bile acid binding resins lower LDL levels, but increase plasma triglyceride levels. *See* Haffner *et al.*, *Diabetes Care*, Vol. 26, Supp. 1 (January 2003) (Evidence 5 in Evidence Exhibit). These publications demonstrate that the methods for lowering plasma lipid levels may not lower and may even increase triglyceride levels. In this regard, not all subjects administered exendins show a decrease in triglycerides, only those in need of such treatment.[1]

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[1] Kim, *et al.*, reported that treatment with exenatide (exendin-4) resulted in significant reductions in triglycerides in subjects having elevated triglycerides prior to the initiation of treatment (baseline triglycerides), but that no significant changes were observed in subjects whose baseline triglycerides were normal. Kim *et al.*, *Diabetic Care*, 23 (Suppl 4) 608-753 Abstract. P1946 (December 2006).

The Office's use of inherency in the obviousness rejection is based on hindsight. The Office has used the knowledge from appellants' disclosure that an exendin or an exendin agonist lowers plasma triglyceride levels as a roadmap to combine Beeley *et al.*'s exendins and exendin agonists with Karpe *et al.*'s subjects having elevated triglyceride levels. Beeley *et al.*'s disclosure of using exendins and exendin agonists to reduce plasma lipid levels or cardiac risk is silent about reductions in triglycerides. The Office has failed to show that one of ordinary skill in the art would have had a reasonable expectation that reducing plasma lipid levels or cardiac risk would result from administering exendins or exendin agonists. Additionally, the Office has not provided objective evidence or reasoning to support that one of ordinary skill in the art would have correlated the reduction of plasma lipid levels or cardiac risk with the lowering of triglyceride levels despite published evidence that decreases in lipids are not necessary correlated with decreases in triglycerides. "[T]o have a reasonable expectation of success, one must be motivated to do more than merely to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful." *Medichem, S.A. v. Rolabo, S.L.*, 437 F.3d 1157, 1165 (Fed. Cir. 2006) (internal quotations omitted). In light of the vast number of treatments for inhibiting food intake and for reducing plasma lipid levels and cardiac risk, combined with the lack of predictability that such treatments would result in lowering triglyceride levels, one of ordinary skill in the art would neither have found it obvious to combine Beeley *et al.*'s methods of administering exendins and exendin agonists with Karpe *et al.*'s subjects at cardiac risk, nor have reasonably expected that such methods would result in lowering triglyceride levels in the subjects.

Accordingly, the claimed subject matter is patentable under 35 U.S.C. § 103 and the rejection should be reversed.

B. Rejection of Claims 15 and 37 under 35 U.S.C. § 103 over Karpe *et al.* and Beeley *et al.*, in view of Wagle *et al.* (US Patent 6,326,396)

For at least the reasons above, Karpe *et al.* and Beeley *et al.* in view of Wagle *et al.* do not teach or suggest identifying a subject with elevated postprandial triglyceride levels and treating

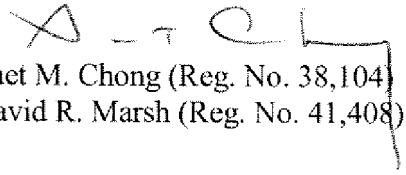
such subjects with exendins or exendin agonists. Wagle *et al.* describes the use of completely unrelated compounds for lipid and glucose lowering. The compounds disclosed in Wagle *et al.* are small molecules while the claims that are the subject of the present appeal are all directed to a particular group of peptides. Whatever else Wagle *et al.* discloses, it too does nothing to remedy the deficiencies of Karpe *et al.* and Beeley *et al.* Additionally the Office has provided no evidence or reasoning why one skilled in the art would combine these references, especially when they address such divergent molecules to arrive at the presently claimed invention.

Accordingly, the claimed subject matter is patentable under 35 U.S.C. § 103 and the rejection should be reversed.

CONCLUSION

In view of the foregoing, Appellants respectfully request that the Board of Patent Appeals and Interferences reverse the pending rejections and that the subject application be allowed forthwith.

Respectfully submitted,



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CLAIMS APPENDIX

1. (Previously Presented) A method for lowering triglyceride levels in a subject in need thereof, comprising:

identifying a subject having elevated postprandial triglyceride levels; and

administering to said subject a therapeutically effective amount of an exendin or an exendin agonist, wherein said subject's postprandial triglyceride levels are lowered.

2. (Previously Presented) The method according to claim 1 wherein said exendin or exendin agonist is administered continuously.

3. (Previously Presented) The method according to claim 1 wherein said administration is by injection.

4. (Previously Presented) The method according to claim 3 wherein the injection is a subcutaneous injection.

5. (Previously Presented) The method according to claim 1 wherein about 1μg to about 1 mg of the exendin or exendin agonist is administered per day.

6. (Previously Presented) The method according to claim 1 wherein about 1μg to about 500 μg of the exendin or exendin agonist is administered per day.

7. (Previously Presented) The method according to claim 1 wherein about 1μg to about 100 μg of the exendin or exendin agonist is administered per day.

8. (Previously Presented) The method according to claim 1, wherein about 3 μg to about 50 μg of the exendin or exendin agonist is administered per day.

9. (Previously Presented) The method of claim 1 wherein said subject is human.

10. (Previously Presented) A method for lowering plasma triglyceride concentrations in a subject with heart disease, comprising:

identifying a patient with heart disease having elevated postprandial triglyceride levels; and

administering to said subject a therapeutically effective amount of an exendin or an exendin agonist, wherein said subject's postprandial triglyceride levels are lowered.

11. (Previously Presented) The method according to any of claims 1-10 wherein said exendin is exendin-3.

12. (Previously Presented) The method according to any of claim 1-10 wherein said exendin is exendin-4.

13. (Previously Presented) The method according to any of claims 1-10 wherein said exendin agonist is selected from the group consisting of exendin-4 acid, exendin-4 (1-30), exendin-4 (1-30) amide, exendin-4 (1-28) amide, ^{14}Leu,^{25}Phe exendin-4, and ^{14}Leu,^{25}Phe exendin-4 (1-28) amide.

14. (Previously Presented) The method according to any of claims 1-10 wherein said exendin agonist is an exendin analog or derivative.

15. (Previously Presented) The method according to any of claims 1-10, further comprising administering a therapeutically effective amount of a statin.

16 - 23. (Canceled)

24. (Previously Presented) A method for lowering postprandial triglyceride levels in a subject in need thereof, comprising:

identifying a subject having elevated postprandial triglyceride levels; and

administering to said subject a therapeutically effective amount of an exendin or an exendin agonist, wherein said subject's postprandial triglyceride levels are lowered.

25. (Previously Presented) The method according to claim 24 wherein said exendin or exendin agonist is administered continuously.

26. (Previously Presented) The method according to claim 24 wherein said administration is by injection.

27. (Previously Presented) The method according to claim 26 wherein the injection is a subcutaneous injection.

28. (Previously Presented) The method according to claim 24 wherein about 1μg to about 1 mg of the exendin or exendin agonist is administered per day.

29. (Previously Presented) The method according to claim 24 wherein about 1μg to about 500 μg of the exendin or exendin agonist is administered per day.

30. (Previously Presented) The method according to claim 24 wherein about 1μg to about 100 μg of the exendin or exendin agonist is administered per day.

31. (Previously Presented) The method according to claim 24, wherein about 3 μg to about 50 μg of the exendin or exendin agonist is administered per day.

32. (Previously Presented) The method of claim 24 wherein said subject is human.

33. (Previously Presented) The method according to any of claims 24-32 wherein said exendin is exendin-3.

34. (Previously Presented) The method according to any of claim 24-32 wherein said exendin is exendin-4.

35. (Previously Presented) The method according to any of claims 24-32 wherein said exendin agonist is selected from the group consisting of exendin-4 acid, exendin-4 (1-30), exendin-4 (1-30) amide, exendin-4 (1-28) amide, ^{14}Leu,^{25}Phe exendin-4, and ^{14}Leu,^{25}Phe exendin-4 (1-28) amide.

36. (Previously Presented) The method according to any of claims 24-32 wherein said exendin agonist is an exendin analog or derivative.

37. (Previously Presented) The method according to any of claims 24-32, further comprising administering a therapeutically effective amount of a statin.

38.-40. (Cancelled)

41. (Previously Presented) The method according to claim 1 wherein said exendin or exendin agonist is selected from the group consisting of exendin-3, exendin-4, exendin-4 acid, exendin-4 (1-30), exendin-4 (1-30) amide, exendin-4 (1-28) amide, ^{14}Leu,^{25}Phe exendin-4, and ^{14}Leu,^{25}Phe exendin-4 (1-28) amide.

EVIDENCE APPENDIX

Exhibit 1: Bravata *et al.*, *JAMA*, Vol. 89, No. 4, pp. 1837-1850 (April 9, 2003)

Exhibit 2: Gaudio *et al.*, *J. Cardiovas. Pharmacol*, Vol. 45, No. 4, pp. 362-366 (April 2005)

Exhibit 3: Ödmark *et al.*, *Maturitas*, Vol. 48, pp. 137-146 (2004)

Exhibit 4: Saklamaz *et al.*, *Metabolism Clinical and Experimental*, Vol. 54, pp. 677-681 (2005)

Exhibit 5: Haffner *et al.*, *Diabetes Care*, Vol. 26, Supp. 1 (January 2003)

EXHIBIT 1

Efficacy and Safety of Low-Carbohydrate Diets
A Systematic Review

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Context Low-carbohydrate diets have been popularized without detailed evidence of their efficacy or safety. The literature has no clear consensus as to what amount of carbohydrates per day constitutes a low-carbohydrate diet.

Objective To evaluate changes in weight, serum lipids, fasting serum glucose, and fasting serum insulin levels, and blood pressure among adults using low-carbohydrate diets in the outpatient setting.

Data Sources We performed MEDLINE and bibliographic searches for English-language studies published between January 1, 1966, and February 15, 2003, with key words such as *low carbohydrate*, *ketogenic*, and *diet*.

Study Selection We included articles describing adult, outpatient recipients of low-carbohydrate diets of 4 days or more in duration and 500 kcal/d or more, and which reported both carbohydrate content and total calories consumed. Literature searches identified 2609 potentially relevant articles of low-carbohydrate diets. We included 107 articles describing 94 dietary interventions reporting data for 3268 participants; 663 participants received diets of 60 g/d or less of carbohydrates—of whom only 71 received 20 g/d or less of carbohydrates. Study variables (eg, number of participants, design of dietary evaluation), participant variables (eg, age, sex, baseline weight, fasting serum glucose level), diet variables (eg, carbohydrate content, caloric content, duration) were abstracted from each study.

Data Extraction Two authors independently reviewed articles meeting inclusion criteria and abstracted data onto pretested abstraction forms.

Data Synthesis The included studies were highly heterogeneous with respect to design, carbohydrate content (range, 0-901 g/d), total caloric content (range, 525-4629 kcal/d), diet duration (range, 4-365 days), and participant characteristics (eg, baseline weight range, 57-217 kg). No study evaluated diets of 60 g/d or less of carbohydrates in participants with a mean age older than 53.1 years. Only 5 studies (nonrandomized and no comparison groups) evaluated these diets for more than 90 days. Among obese patients, weight loss was associated with longer diet duration ($P=.002$), restriction of calorie intake ($P=.03$), but not with reduced carbohydrate content ($P=.90$). Low-carbohydrate diets had no significant adverse effect on serum lipid, fasting serum glucose, and fasting serum insulin levels, or blood pressure.

Conclusions There is insufficient evidence to make recommendations for or against the use of low-carbohydrate diets, particularly among participants older than age 50 years, for use longer than 90 days, or for diets of 20 g/d or less of carbohydrates. Among the published studies, participant weight loss while using low-carbohydrate diets was principally associated with decreased caloric intake and increased diet duration but not with reduced carbohydrate content.

JAMA. 2003;289:1837-1850 www.jama.com

BETWEEN 1960 AND 2000 THE prevalence of obesity among adults aged 20 years to 74 years in the United States increased from 13.4% to 30.9%.[1-3] An estimated 325 000 deaths and between 4.3% and 5.7% of direct health care costs (approximately $39-$52 billion) are attributed to obesity annually.[1,2] Results from the 1998 Behavioral Risk Factor Surveillance Survey indicate that roughly one third of US adults were trying to lose weight at that time, and another third were trying to maintain weight.[4] Recently, low-carbohydrate diets have resurged in popularity as a means of rapid weight loss, yet their long-term efficacy and safety remain poorly understood.

The first low-carbohydrate diet to have enjoyed popular success was that described by William Banting in the 1860s.[5] Banting claimed that he was never hungry and at the age of 66, in a period of a year, lost 46 of his initial 202 pounds. He wrote, "The great charms and comfort of the system are that its effects are palpable within a week of trial and creates a natural stimulus to persevere for a few weeks more."[5]

While it is difficult to estimate the number of people who have followed low-carbohydrate diets, the number and popularity of articles and books from the lay press advocating their use attest to a high level of interest in and demand for these diets by the US public. The most popular text, written by cardiologist and long-time proponent

For editorial comment see p 1853.

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of low-carbohydrate diets Robert Atkins, has been on the *New York Times* bestsellers' list continuously for more than 5 years.[6] Over the past 5 years, 3 books on low-carbohydrate diets collectively sold millions of copies in the United States.[6-8] Advocates of low-carbohydrate diets claim that diets higher in protein and lower in carbohydrates promote the metabolism of adipose tissue in the absence of available dietary carbohydrate and result in rapid weight loss without significant long-term adverse effects.[6]

However, numerous professional organizations, including the American Dietetic Association and the American Heart Association, have cautioned against the use of low-carbohydrate diets.[9-12] There are concerns that low-carbohydrate diets lead to abnormal metabolic functioning that may have serious medical consequences, particularly for participants with cardiovascular disease, type 2 diabetes mellitus, dyslipidemia, or hypertension. Specifically, it has been cautioned that low-carbohydrate diets cause accumulation of ketones and may result in abnormal metabolism of insulin and impaired liver and kidney function; in salt and water depletion that may cause postural hypotension, fatigue, constipation, and nephrolithiasis; in excessive consumption of animal proteins and fats that may promote hyperlipidemia; and in higher dietary protein loads that may impair renal function.[13,14]

The medical literature pertaining to the efficacy and the metabolic effects of low-carbohydrate diets is composed of numerous heterogeneous studies of relatively few participants. The studies vary in terms of dietary interventions provided (eg, percentage of calories from carbohydrate, fat, and protein), type of participants enrolled (eg, participants with diabetes or with hyperlipidemia), and outcomes evaluated (eg, weight loss or change in glycemic control). The purpose of this study was to synthesize the literature on low-carbohydrate diets to evaluate changes in weight, serum lipid, fasting serum glucose, and fasting serum insulin levels, and blood pressure among adults using low-carbohydrate diets in the outpatient setting.

Table 1. Results of Literature Search

Description	No. of Articles
MEDLINE key word searches	
Search 1, *diet**	192 654
Search 2, *low carbohydrate**	567
Search 3, *high fat**	5782
Search 4, *high protein**	3473
Search 5, *ketogenic*	706
Search 6, *isocaloric*	2808
Search 7, *hypocaloric*	706
Search 8, *protein sparing*	2014
Search 9, *carbohydrate restricted*	6362
Combine searches: 1 AND (2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9) LIMIT by (participant types: adults and humans) then discard duplicates	2609
Exclusion criteria	
Articles included only pediatric participants (no adults)	21
Diet duration <4 d	95
Did not report sufficient data to be able to calculate grams per day of carbohydrates per diet	72
Did not report sufficient data to be able to calculate calories per day per diet	97
Participants hospitalized or confined to a research center	146
Diets that provided <500 kcal/d	26
Review article	36
Article not in English	17
Included only pregnant participants	5
Did not report data for any of the outcomes of interest	1994
Total articles excluded from those found in the MEDLINE search†	2509
Articles included from manual searches of bibliographies	7
Total articles included in the analyses	107
Combine duplicated reports on the same study participants	13
Total studies of dietary interventions included	94

*All terms beginning with this root would be included in the search (eg, searching with the root *diet* includes terms such as *diets*, *dieting*, and *dietary*).
†Several articles met more than 1 of the exclusion criteria.

METHODS

Data Sources

Two authors and a professional librarian independently developed search strategies to identify studies that met the eligibility criteria. We performed searches on MEDLINE for English-language studies published between January 1, 1966, and February 15, 2003, that were indexed with key words including *diet*, *low carbohydrate*, *high fat*, *high protein*, and *ketogenic* (TABLE 1). We also reviewed the bibliographies of retrieved articles and conference proceedings to obtain additional citations.

Study Selection Criteria

English-language studies were considered eligible for this analysis if they evaluated any of the following interventions: low-carbohydrate, ketogenic, higher-protein, or higher-fat diets for adults who were not pregnant. Additionally, the included studies had to report sufficient data to calculate both carbohydrate content (grams per day) and total calories consumed (kilocalories per day). Because we were interested in diets that could be followed by outpatient adults, studies that evaluated diets with the following characteristics were excluded: less than 500 kcal/d, duration of diet less than 4 days, or requirement for participants to be hospitalized or confined to a research or diet center. Articles were excluded if they did not report data for at least 1 of the clinical outcomes of interest.

Abstraction Methods

One author reviewed the 2609 titles and abstracts identified by the combined

MEDLINE search for potentially relevant studies. Two authors independently abstracted study design and participant data onto pretested abstraction forms from each of these publications and reviewed bibliographies for additional potentially relevant studies. Abstraction discrepancies were resolved by repeated review and discussion. If 2 or more studies presented the same data from a single participant population, these data were included only once in the analyses. If a study presented data on 2 types of diets and if 1 of the diets did not meet our inclusion criteria (eg, studies that compared a fast- with a lower-carbohydrate diet), then data were abstracted only for those participants receiving the diet that met the inclusion criteria.

Data Abstracted

Three types of variables were abstracted from each study: dietary intervention, participants studied, and clinical outcomes. The variables for dietary intervention abstracted were carbohydrate, fat, and protein content (grams/day), daily caloric content (kilocalories per day), and the duration of dietary intervention (days). The participant variables abstracted were type of participants enrolled (eg, athletes, healthy volunteers, or participants with obesity, hyperlipidemia, diabetes, or hypertension), age, sex, and race/ethnicity. The outcome variables abstracted were measures of body mass (weight in kilograms, body mass index [BMI] calculated as weight in kilograms divided by the square of height in meters, and percentage of body fat), measures of lipid levels (total cholesterol, low-density lipoprotein [LDL] cholesterol, high-density lipoprotein [HDL] cholesterol, and serum triglycerides), measures of glycemic control (fasting serum glucose and insulin levels), and a measure of hypertensive control (systolic blood pressure).

Statistical Analyses

For each study, a weighted mean was calculated for each of the participant and diet characteristics (weighted by the number of participants who completed the study). We calculated an effect size for each outcome variable for each study (ie, standardized mean difference) from the mean change in the variable from the start of the diet to the end of the diet and the variance about this change.[15] If the study did not report these data, a pooled variance was calculated.[15] If an individual study did not report a measure of variance for the start or the end values of each outcome variable, then a weighted mean variance was calculated, and this weighted mean variance was used to calculate the pooled variance.[15] If a study did not report any measure of variance for an outcome, the overall mean pooled variance was used for that study.

Typically, a meta-analysis is the quantitative synthesis of independent studies, each of which was designed to compare the effects of a standard treatment with an experimental treatment. Because the studies are independent, so are the effect sizes. In the case of our analysis, the participant, diet, and outcome variables for each participant studied are correlated; therefore, the corresponding estimated effect sizes for these measures are correlated.[16] Multivariate analysis of variance (weighted by the number of participants who finished each diet) was used to calculate the summary effect of the dietary and participant characteristic variables on the outcome variables.[15] For example, using this method, the effect of carbohydrate content controlled for diet duration and calorie content on weight loss could be determined.

Bivariate analyses were performed to estimate the differences in weight loss, serum lipid, fasting serum glucose, and fasting serum insulin levels, and blood pressure between participants who were grouped into 2 general categories of lower- vs higher-carbohydrate diets. The bivariate analyses required setting a threshold to classify lower- vs higher-carbohydrate diets. Because the literature has no clear consensus as to what amount of carbohydrates per day constitutes a low-carbohydrate diet, the differences between lowest-, lower-, and higher-carbohydrate diets by carbohydrate thresholds of 20 g/d or less, 60 g/d or less, and more than 60 g/d, respectively, were evaluated. Unless otherwise specified, these thresholds will be used to define these categories of low-carbohydrate diets. These thresholds correspond to recommendations found in the popular literature of low-carbohydrate diets.[6-8]

For the bivariate analyses, effect sizes were combined using a fixed effects model, which produces a narrower 95% confidence interval (CI), thereby increasing the likelihood of finding a difference between lower- and higher-carbohydrate diets. Then tests of homogeneity on summary effect sizes using the Q statistic were calculated. We attempted to minimize multiple comparisons. Because 4 outcome groups of interest (changes in body mass, serum lipid levels, glycemic indicators, and blood pressure) were included, a Bonferroni adjustment was used and the null hypothesis was rejected only if the level of significance was less than .0125 (.05/4=.0125). Analyses were performed using SAS version 6.12 (SAS Institute, Cary, NC), SPSS version 9.0 (SPSS Inc, Chicago, Ill) and Microsoft Excel 2000 (Microsoft Corp, Redmond, Wash).

RESULTS

Identified Studies

Our MEDLINE search identified 2609 titles of potentially relevant articles. We obtained 7 additional references from manual searches of the bibliographies of retrieved articles (Table 1). A total of 107 articles met the inclusion criteria. After combining multiple reports on the same study participants, we included 94 dietary interventions (Table 1 and TABLE 2).

Study Characteristics

The designs of the included studies were highly heterogeneous (Table 2). Several studies included a washout phase at the beginning of the study interval, during which participants typically received a standard or maintenance diet that was intended to simulate their usual diet in calories and macro-

nutrient composition. For those studies including a washout phase, we considered the participants' weight at the end of the washout phase as their baseline weight.

Forty-three studies used randomized research designs: 24 studies were randomized controlled trials[17-46] in which participants were randomized to receive 1 of 2 or more diets and 19 studies were randomized crossover trials[47-65] in which participants were randomized to receive one diet first and then to receive a second diet. Of those studies that did not use randomized research designs, 17 studies[66-84] compared participants receiving a lower-carbohydrate diet with a comparison group receiving an alternative diet. In some studies, participants were allowed to decide which diet they would prefer to maximize adherence to the prescribed diet. Nine studies[85-94] had a sequential design in which

Table 2. Characteristics of Low-Carbohydrate Studies by Study Design*

Source	Total No. of Participants†	Age, Mean (Range), y	Sex, % Male	Duration of Diet, d	No. of Arms in Study†	Dietary Composition, Range			
						Carbohydrates, g	Proteins, g	Fats, g	Total Calories, kcal
Randomized Controlled Trials									
Vessby et al[17]	162	49 (30-65)	53	90	2	236-247	80-81	88-89	2140-2150
Hockaday et al[18]	93	52 (22-65)	56	365	2	150-203	75	43-67	1500
Shah et al[19]	89	36 (25-45)	0	180	2	225-242	63-66	49-53	1684-1740
Lean et al[20]	82	51 (18-68)	0	180	2	110-186	62-89	27-47	1197-1198
Baron et al[21]‡	63	40	15	90	1	50	NA	NA	1200
Kratz et al[22]	58	26 (18-43)	0-100	28	6	272-304	79-92	100-110	2318-2574
Saltzman et al[23,24]	43	45	43-50	42	2	229-234	79-82	67-69	1827-1872
Schlundt et al[25]	49	44	13	140	2	179-210	61-64	28-30	1265-1426
Foster et al[26]‡	47	41	0	84	2	30-100	70-90	13-20	660-800
Skov et al[27-29] and Hautvik et al[30]	46	40 (18-56)	23	180	2	248-386	79-131	70-83	2139-2605
Heilbronn et al[31]	45	NA (56-58)	46-57	56	2	212-218	78-80	27-29	1436-1442
Helge[32]	41	27	100	49	3	184-566	127-149	79-245	3370-3561
Scott et al[33]	36	38 (29-49)	0	56	2	104-152	51-53	22-46	1003-1005
Brussaard et al[34]	35	NA (19-30)	66	94.5	2	315-375	93-98	57-85	2460-2470
Wolever and Mehling[35]	34	56 (30-65)	18-23	112	3	223-232	75-82	47-74	1695-1879
Brown et al[36]	32	26 (16-62)	94	84	2	359-631	114-124	50-196	3378-3670
Luscombe et al[37]	26	NA (52-64)	33-55	56	2	167-219	64-112	46-49	1583-1585
Fagerberg et al[38]	23	50 (44-56)	100	28	2	81-204	69-70	26-72	1370-1400
Kogon et al[39]	23	40 (23-55)	0	28	2	29-116	69	23-62	950
Racette et al[40]	23	39 (21-47)	0	84	4	76-182	32-74	69-74	1147-1231
Hammer et al[41]§	14	33	0	112	1	114	50	16	800
Mathieson et al[42] and Walberg et al[43]	12	NA (23-36)	0	28	2	44-94	33	3-25	530
Foster et al[44] and Wadden et al[45]‡	9	41	0	336	1	146	60	41	1194
Coyle et al[46]	7	25	100	14	2	718-901	113-117	9-105	4358-4444
Randomized Crossover Trials									
Parker et al[47]	54	61	35	56	2	167-211	63-111	45-49	1543-1587
Miller et al[48]	43	54	43	56	1	289	95	63	2100
Peterson and Jovanovic-Peterson[49]	25	36 (21-50)	0	42	1	150	NA	NA	1500
Muller et al[50]	25	31	0	20-22	3	243-330	77-85	45-89	2069-2115
vanStratum et al[51]	22	53	0	14	2	174-287	90	53-102	1970-1987
Luscombe et al[52]	21	57	67	28	2	202-272	106-110	77-42.4	1910-1924
Rosen et al[53]	20	29 (20-38)	5	14	2	0-50	77-105	33-55	800-917
Jenkins et al[54]	20	56 (35-71)	75	30	2	323-415	111-189	78-80	2764-2835
Weinsier et al[55]‡	18	59 (43-69)	61	112	2	190-264	69-74	56-90	1847-1849
Simpson et al[56]	14	54	83	42	2	209-375	98	63-137	2458-2462
Straznicky et al[57]	14	26	100	14	2	250-301	106-109	60-136	2187-2636
Pomerleau et al[58]	12	58	67	21	2	299-302	52-125	56-80	2103-2175
Lousley et al[59]	11	64 (51-75)	NA	42	2	115-202	67-70	23-60	1240
Wolfe and Piche[60]	10	28 (20-57)	20	28	2	258-304	84-116	84-85	2155-2178
Wolfe and Giovannetti[61]	10	50 (24-67)	40	28	2	263-344	52-108	52-53	1909-2011
Whitehead et al[62]	8	49 (31-57)	25	7	3	80-133	38-90	36-59	1004
Carey et al[63]	7	24	100	6	2	183-767	183-185	82-346	4584-4629
Holmback et al[64]	7	32 (26-43)	100	6	2	299-485	112	66-149	2988
Spaulding et al[65]	6	NA (25-43)	NA	14	4	0-200	0-55	0-71	600-1066

(continued)

Table 2. Characteristics of Low-Carbohydrate Studies by Study Design* (cont)

Source	Total No. of Participants†	Age, Mean (Range), y	Sex, % Male	Duration of Diet, d	No. of Arms in Study†	Dietary Composition, Range			
						Carbohydrates, g	Proteins, g	Fats, g	Total Calories, kcal
Trials With Control or Comparison Group									
Bialkowska et al[66]	101	NA	0	42	2	75-113	55-71	43-73	1066-1237
Fleming[67]	100	43 (23-67)	47	365	4	36-315	51-100	15-97	1350-2100
Golay et al[68]	68	45	22	84	2	70-132	85-86	34-57	1142-1179
Luntz and Reuter[69]	61	54 (27-87)	34	180	2	100	NA	NA	1000
Mezzano et al[70]	42	22	100	90	2	207-269	86	59-87	1952
Afford et al[71]	35	39 (31-56)	0	70	3	75-225	45-90	13-60	1200
Hellbronn et al[72]	35	58	23	84	3	198-280	65-73	17-57	1541-1613
Donnelly et al[73]	35	NA	0	90	3	79	50	1	525
Thompson et al[74]‡	27	29	100	14	2	183-668	116-126	77-296	3856-3871
Volek et al[75] and Sharman et al[76]	20	36.7	100	42	2	46-283	80-176	56-157	1950-2335
Ireland et al[77]	18	30	38	14	2	87-124	96-250	28-111	1617-1887
Gumbiner et al[78] and Low et al[79]	17	53	47	42	2	39-312	84-87	21-127	1635-1785
Vaswani[80]	17	31	0	84	2	10-70	NA	NA	800
Young et al[81]	8	23 (21-28)	100	63	3	30-104	115	103-135.5	1800
Simonyi et al[82]	7	28-47	0-100	6-10	7	15-25	NA	NA	1537-2010
Greenhaff et al[83]	6	28	100	4	3	20-486	100-174	36-207	2622-2673
Greenhaff et al[84]	5	31	100	4	2	22-617	75-179	29-242	2988-3011
Sequential Study Design									
Hulley et al[85]	41	NA (24-59)	100	180	2	160-277	97-102	114-117	2100-2523
Pieke et al[86]	19	39.2 (28-58)	100	14-28	2	242-312	101-105	71-102	2313-2390
Bonanome et al[87]	19	55 (40-61)	53	60	3	182-242	57-61	42-72	1518-1616
Serog et al[88] and Apfelbaum et al[89]	18	NA (18-22)	NA	NA	2	70-525	105	31-233	2800
Hallak et al[90]	16	NA (18-30)	100	14	2	160-327	59-64	17-104	1696-1834
O'Dea et al[91]	10	61 (50-69)	100	14	4	91-292	95-246	19-129	1586-2115
Cattran et al[92]	8	34 (24-57)	63	21	3	92-242	70-84	40-117	1638-1760
Ekstedt et al[93]	7	NA (21-37)	100	8	4	323-542	109-162	54-220	2300-3800
Fery et al[94]‡	6	NA (22-46)	NA	4	1	25	101	159	2026
Pre-Post Studies									
Nobels et al[95]	113	37	NA	180	1	38	84	29	750
Rabast et al[96]	104	37	33	106	1	40	60	100	1340
Kirby et al[97]	59	NA (18-70)	20	126	1	30	NA	NA	1000
Bettens et al[98]	57	41	21	60	1	40	NA	NA	1200
Calle-Pascual et al[99]	54	43	15	140	1	113	73	54	1260
Conti et al[100]	46	52 (40-60)	57	30	1	211	63	40	1400
Harvey et al[101]	42	52	38	180	1	180	45	40	1260
Westman et al[102]	41	44	24	168	1	23	115	98	1447
Spiller et al[103]	26	56 (29-81)	NA	63	1	241	103	90	2194
Mogul et al[104]‡	26	47, 54	0	365	2	159	122	38	1500
Krotkiewski et al[105]	25	40	0	28	1	65	55	7	544
Larosa et al[106]	24	NA (20-58)	58	56	1	6	107	108	1461
Engelhart et al[107]	19	NA (34-71)	15	84	1	193	112	45	1625
De Lorenzo et al[108]	19	32	0	60	1	214	78	43	1554
Serog et al[87] and Apfelbaum et al[89]	14	NA (18-22)	10	14	1	36	70	16	560
Marsoobian et al[109]	13	NA (18-28)	0	14	1	30	NA	NA	600
Cangiano et al[110]	10	43	0	42	1	245	85	87	2066
Volek et al[111,112]	10	26	100	56	1	39	147	151	2110
Buffenstein et al[113]	9	NA (20-36)	0	28	1	102	41	15	743
Cordera et al[114]	8	37	100	60	1	165	98	50	1500
Evans et al[115]	8	NA (21-40)	0	42	1	86	75	94	1490
Benoit et al[116,117] and Grande et al[118]	7	29 (22-45)	100	10	1	10	35	91	1000
Staudacher et al[119]‡	7	24	100	6	1	197	171	351	4626
Kwan et al[120]	6	21 (20-23)	0	7	1	49	103	164	2066
Elliot et al[121]	2	23 (22-23)	50	24	1	7	180	225	2773

Abbreviation: NA, not available.

*Study and design: Randomized controlled trials were those in which participants were randomized to receive 1 of 2 or more diets; randomized crossover trials were those in which participants were randomized to receive one diet first and then to receive a second diet. Sequential study designs were those in which all participants received 2 or more diets in the same order; pre-post studies were those in which a single group received a single diet.

†Total number of participants completing the diets. Number of arms equals number of diets evaluated.

‡Only data for participants receiving dietary interventions meeting the exclusion criteria were included.

Table 3. Diet Characteristics

	Carbohydrates in Diet, g/d						
	Lower, ≤60			Higher, >60			
	No. of Diets	Mean (SD)	Median (Range)	No. of Diets	Mean (SD)	Median (Range)	*P* Value
Carbohydrate content, g/d	38	29 (15)	30 (0-60)	157	236 (141)	211 (65-901)	<.001
Protein content, g/d	26	96 (45)	95 (33-180)	150	89 (36)	83 (0-250)	.30
Fat content, g/d	26	104 (65)	99 (16-242)	150	69 (58)	57 (0-351)	.01
Caloric content, kcal/d	38	1446 (653)	1454 (530-2988)	157	1913 (880)	1740 (525-4629)	.002
Diet duration, d	37	50 (70)	24 (4-365)	152	73 (83)	42 (4-365)	.10

all participants received 2 or more diets in the same order. Twenty-five studies[95-121] were pre-post evaluations in which a single group received a single diet. For those participants in studies with randomized crossover and sequential diet designs, we did not use the data from the second diet interval in our analyses because participants did not typically return to their baseline weight between diets.

Diet Characteristics

The included studies reported on 38 lower-carbohydrate diets,* (≤ 60 g/d of carbohydrates); 13† of these 38 were lowest-carbohydrate diets (≤20g/d of carbohydrates). Lower-carbohydrate diets had lower caloric contents (mean, 1446 kcal/d) than higher-carbohydrate (>60 g/d of carbohydrates) diets (mean, 1913 kcal/d, $P=.002$). Studies of lower-carbohydrate diets tended to have a shorter duration than studies of higher-carbohydrate diets (mean, 50 days and mean, 73 days, respectively; $P=.10$) (TABLE 3). Studies of the lowest-carbohydrate diets had shorter duration (mean [range], 19 [4-84] days) than the lower- and higher-carbohydrate diets ($P=.02$). Only 5 studies evaluated lower-carbohydrate diets for more than 90 days, and these studies were nonrandomized and noncontrolled designs (Table 2).[67,95-97,102]

All of the studies in our systematic review included participants in the outpatient setting. The studies used a variety of methods to verify that the participants adhered to the prescribed diet. These methods included food diaries, measured ketonuria or serum β-hydroxybutyrate levels, comparison of the expected sodium intake with observed urinary sodium levels, and multiple or no verification methods.

Because most weight loss programs include both diet and exercise, we were interested in comparing lower-carbohydrate diets with and without exercise. However, the included studies varied significantly with respect to the amount of description of the exercise component. For example, many studies simply stated that exercise was encouraged but did not present information about the type, frequency, or duration of exercise by participants. Therefore, given the lack of sufficiently detailed data, we excluded exercise information from our analyses.

Participant Characteristics

The included studies present data on 3268 participants who completed the diets: 663 participants received lower-carbohydrate diets, of whom only 71 received lowest-carbohydrate diets (TABLE 4). No significant difference was found in the age or sex of recipients of lower- vs higher-carbohydrate diets. The mean (SD) age of recipients of lower-carbohydrate diets was 37.6 (8.5) years and no study of lower-carbohydrate diets had a mean age older than 53.1 years. The participants' weight before diet, BMI, percentage of body fat, serum lipid, fasting serum glucose, and fasting serum insulin levels, and systolic blood pressure did not differ significantly between the lower- and higher-carbohydrate groups (Table 4). The definitions of what constituted a healthy volunteer, obese participant, or participant with diabetes varied among studies. The classifications of racial/ethnic groups also varied among studies that reported data on race/ethnicity; thus, these classifications were not included in our analyses.

Effect of Diet and Participant Characteristics on Efficacy and Safety Variables

Results of the bivariate analyses compare the differences in each of the outcome variables between recipients of lower- vs higher-carbohydrate diets (TABLE 5). The interpretation of these analyses is complicated by the significant heterogeneity of the included studies. For example, because the included diets were not isocaloric, the lower-carbohydrate diets vary significantly with respect to the percentage of calorie intake from carbohydrates. We have attempted to compare diets with similar caloric contents, durations, and study designs to account for this heterogeneity.

Change in Weight, BMI, and Percentage of Body Fat. At the end of both lower- and higher-carbohydrate diets, participants' weight, BMI, and percentage of body fat decreased (Table 5). In general, for both lower- and higher-carbohydrate diets, we found the greatest weight loss occurred among those participants receiving diets with the lowest caloric content and for those participants with the highest baseline weights (Table 5).

*References 21, 26, 39, 42, 43, 53, 65, 67, 75, 76, 78-80, 82-84, 88, 89, 94-98, 102, 106, 109, 111, 112, 116-118, 121.
†References 53, 65, 80, 82, 83, 106, 116-118, 121.

The 72 young participants of the 14 diets* of very short duration (<15 days) receiving lower-calorie diets (mean [SD] age, 26.8 [8.5] years; mean [SD], 23 [13] g/d of carbohydrates; mean [SD], 1597 [715] kcal/d for participants with a mean [SD] weight before diet of 78.4 [5.2] kg) demonstrated significant mean [SD] weight loss (13.6 [0.1] kg); however, no data were available about whether they maintained this weight loss beyond the study period.

Of the 34† of 38 lower-carbohydrate diets for which weight change after diet was calculated, these lower-carbohydrate diets were found to produce greater weight loss than higher-carbohydrate diets (absolute summary mean [SD] change, 16.9 [0.2] kg; 95% CI, 16.6-17.3 kg vs 1.9 [0.2] kg; 95% CI, 1.6-2.2 kg) (Table 5). Because the 95% CIs for the lower- and higher-carbohydrate diets do not overlap, it suggests that a difference may exist in weight change between the 2 types of diets. However, the highly heterogeneous nature of the 34 diets is reflected in the significant Q statistic associated with the summary mean changes in weight calculated when all studies were included in the analysis.

*References 53, 82, 84, 88, 89, 109, 116-118, 120.

†References 21, 26, 39, 42, 43, 53, 67, 75, 76, 78-80, 82, 84, 88, 89, 95-98, 102, 106, 109, 111, 112, 116-118, 121.

Table 4. Participant Characteristics Before and After Diet

	Carbohydrates in Diet, g/d								
	Lower, ≤60				Higher, >60				
	No. of Diets	No. of Participants	Mean (SD)*	Median (Range)	No. of Diets	No. of Participants	Mean (SD)*	Median (Range)	*P* Value
Age, y	38	692	37.6 (8.5)	35 (20-53.1)	147	2605	44.3 (12.6)	39.6 (20-64.2)	.90
Sex, % male	34	561	30 (43)	29 (0-100)	131	2483	42.0 (40.0)	46 (0-100)	.60
Weight, kg									
Before diet	23	568	91.7 (15.8)	87 (57.2-115.6)	118	2247	86.2 (19.7)	81.4 (61-217)	.90
After diet	18	435	79.3 (10.1)	77.5 (55.5-94.5)	113	1844	82.8 (18.9)	77.6 (60.1-210)	.30
BMI, kg/m^2									
Before diet	3	145	36.3 (5.2)	36.3 (36.0-37.5)	36	925	30.6 (4.1)	29.2 (21.8-39.7)	.05
After diet	1	113	29.7 (4.1)	29.7 (29.7-29.7)	28	739	28.0 (3.5)	26.3 (21.7-35.0)	.50
Percentage of body fat, %									
Before diet	5	76	38.1 (6.2)	31.4 (20.5-44)	33	655	37.2 (4.5)	39.0 (12.8-47.3)	.70
After diet	5	66	33.9 (5.0)	22.3 (16.9-41)	27	536	33.2 (4.9)	33.2 (12.2-39.8)	.60
Cholesterol, mg/dL									
Total									
Before diet	13	227	191.1 (21.2)	186 (148.2-214)	79	1519	246.4 (42.5)	201 (124-267.8)	.03
After diet	12	205	188.3 (29.4)	186 (119.6-348)	75	1322	201.8 (36.1)	197 (136.6-252.5)	.60
LDL									
Before diet	7	181	118.6 (20.7)	119.9 (103.6-136)	43	934	137.4 (30.9)	129 (86.5-212.7)	.20
After diet	7	168	123.1 (20.7)	116.6 (96.7-151)	42	852	130.2 (20.2)	127.9 (47-189.5)	.60
HDL									
Before diet	10	197	51.3 (12.7)	49.1 (27.1-87)	48	1080	48.7 (13.4)	47.3 (30.9-72.8)	.60
After diet	9	175	53.3 (8.1)	53.0 (37.1-87)	48	984	48.4 (9.9)	46.4 (27.1-77.3)	.20
Triglycerides, mg/dL									
Before diet	13	227	136.5 (60.8)	115 (68.7-283.4)	74	1674	138.3 (53.4)	129.6 (47.8-377.1)	.50
After diet	13	214	98.1 (38.7)	93.0 (57.9-130.2)	70	1245	126.2 (46.8)	123 (50-247.1)	.01
Fasting serum glucose, mg/dL									
Before diet	11	252	101.3 (11.1)	95.0 (73.8-226.8)	60	1040	130.5 (37.1)	97.2 (72.5-225)	.90
After diet	11	249	91.4 (19.3)	87.0 (68-144)	59	871	112.4 (24.6)	99 (57.5-205.2)	.10
Fasting serum insulin, μIU/mL									
Before diet	6	55	10.2 (4.7)	10.2 (3.4-16.4)	44	839	10.3 (8.5)	10.3 (1.0-36.0)	.90
After diet	6	55	6.6 (2.6)	6.3 (2.2-10.2)	46	778	9.4 (4.3)	7.9 (0.98-38.0)	.50
Systolic blood pressure, mm Hg									
Before diet	3	132	138.9 (16.2)	126.0 (112-141.9)	23	507	134.6 (16.7)	133 (111-148)	.50
After diet	3	132	125.1 (12.6)	119.0 (107.7-126.8)	20	403	127.4 (12.3)	129.6 (105-136)	.20

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

SI conversion factors: To convert mg/dL to mmol/L for total cholesterol, LDL, and HDL, multiply by 0.0259. To convert mg/dL to mmol/L for triglycerides, multiply by 0.0113. To convert mg/dL to mmol/L for fasting serum glucose, multiply by 0.0555. To convert μIU/mL to pmol/L for fasting serum insulin, multiply by 6.945.

*Means are weighted by the number of participants (eg, mean BMI before diet is weighted by the number of participants starting the diet and the mean BMI after diet is weighted by the number of participants completing the diet). Because the studies used to calculate the data before and after diet often differ, the change in outcomes should not be interpreted as the difference between the means before and after the diet (data reflecting the summary mean changes in outcomes are presented in Table 5).

Given this heterogeneity, little can be concluded about the summary mean change in weight loss when all studies are combined. When only the randomized controlled trials and the randomized crossover trials in the analysis are included, the result of the Q statistic suggests that the studies are homogeneous. From this selected group of relatively similar randomized studies of 7 lower-carbohydrate diets[21,26,39,42,43,53] and 75 higher-carbohydrate diets we found

Table 5. Summary Mean Change in Outcomes*

	Carbohydrates in Diet, g/d							
	Lower, ≤60				Higher, >60			
	No. of Diets†	No. of Participants	Summary Mean Change‡ (SD)	95% CI	No. of Diets	No. of Participants	Summary Mean Change‡ (SD)	95% CI
Weight change, kg								
All studies, all participants	34	668	−16.9 (0.2)§	−16.6, −17.3	130	2092	−1.9 (0.2)§	−1.6, −2.2
RCT and R-Cross only	7	132	−3.6 (1.2)	−1.2, −6.0	75	1122	−2.1 (0.3)	−1.6, −2.7
Caloric content of the diet, kcal/d								
<1500	18	614	−17.5 (0.2)§	−17.1, −17.8	45	870	−3.1 (0.4)§	−2.4, −3.8
≥1500	16	53	−5.7 (0.2)§	−5.4, −6.0	84	1222	−1.5 (0.2)§	−1.2, −1.9
Diet duration, d								
<15	14	72	−13.6 (0.1)§	−13.5, −13.8	25	198	−1.5 (0.2)§	−1.1, −1.8
16-60	9	142	−5.3 (0.6)§	−4.2, −6.4	52	827	−3.5 (0.4)§	−2.9, −4.3
>60	10	447	−2.4 (2.1)	+1.8, −6.5	45	968	−1.1 (0.6)	−.01, −2.3
Participant age, y								
<40	22	426	−17.7 (0.2)§	−17.4, −18.1	59	642	−1.4 (0.2)§	−1.0, −1.8
≥40	12	242	−5.0 (0.6)§	−3.8, −6.2	62	1231	−2.9 (0.3)§	−2.4, −3.5
Baseline weight, kg								
<70	3	22	−19.6 (0.2)§	−19.2, −20.0	19	230	−3.2 (0.6)§	−1.9, −4.4
70-100	13	365	−0.8 (1.6)	+2.4, −4.0	77	1357	−2.4 (0.4)	−1.3, −0.4
>100	7	138	−6.6 (0.7)§	−5.2, −8.0	18	301	−8.1 (0.8)§	−6.5, −9.7
BMI, kg/m²								
All studies, all participants	1	113	−1.4 (4.6)	+7.6, −10.3	27	739	−0.4 (0.4)	+0.3, −1.1
Body fat, %								
All studies, all participants	5	66	−1.0 (5.6)	+4.0, −6.0	27	536	−1.0 (0.6)	+0.1, −2.1
Cholesterol, mg/dL								
Total								
All studies, all participants	13	214	−1.2 (7.3)	+13.2, −15.5	87	1633	−8.1 (1.4)	−5.5, −10.8
RCT and R-Cross only	3	77	−1.9 (9.7)§	+17.1, −20.8	43	903	−1.4 (3.3)	+5.0, −7.9
LDL								
All studies, all participants	7	168	−0.3 (9.7)	+19.3, −18.7	42	852	−0.7 (3.1)	+5.3, −6.8
RCT and R-Cross only	1	63	+0.4 (30.7)	+60.5, −59.7	22	563	−1.0 (3.7)	+6.3, −8.3
HDL								
All studies, all participants	9	175	−0.2 (2.1)	+4.0, −4.3	46	964	−0.8 (0.6)	+0.4, −2.0
RCT and R-Cross only	3	77	−0.8 (4.2)	+7.5, −9.1	22	553	−0.9 (0.7)	+0.4, −2.3
Triglycerides, mg/dL								
All studies, all participants	13	214	+4.1 (4.5)	+13.0, −4.6	78	1531	−0.6 (3.3)	+7.1, −6.0
RCT and R-Cross only	3	77	+0.3 (19.0)	+37.6, −37.0	43	903	−1.3 (4.4)	+9.9, −7.4
Fasting serum glucose, mg/dL								
All studies, all participants	11	249	−1.3 (2.8)	+4.3, −6.8	59	871	−0.4 (1.2)	+1.9, −2.7
RCT and R-Cross only	2	69	−0.3 (27.4)	+53.4, −54.0	17	455	−0.3 (1.3)	+2.4, −3.0
Fasting serum insulin, pmol/L								
All studies, all participants	5	45	−0.8 (9.9)	+18.5, −20.1	44	764	−0.4 (1.6)	+2.9, −3.7
RCT and R-Cross only	0	...	...	...	26	467	−0.01 (2.3)	+4.4, −4.5
Systolic blood pressure, mm Hg								
All studies, all participants	4	173	0.7 (5.2)	+10.8, −9.5	25	481	0.6 (2.5)	+5.6, −4.3

Abbreviations: BMI, body mass index; CI, confidence limits; ellipses, insufficient data to calculate outcome; HDL, high-density lipoprotein; LDL, low-density lipoprotein; RCT, randomized controlled trial; R-Cross, randomized crossover trial.

*See Table 4 for the conversion of conventional units to SI units.

†The reason that the number of diets and number of participants for whom we were able to calculate a difference in each of the outcomes is greater than the number of diets and number of participants for whom we presented in the data before and after diet (Table 4) is that some studies reported only the change in the outcome but not before or after diet data.

‡Summary mean change in each outcome variable was calculated from a standardized mean difference. A negative change in any of the outcome variables denotes a reduction in that variable after the diet interval. For example, the absolute summary mean change in weight loss calculated from all studies of lower-carbohydrate diets was 16.9 kg.

§The Q statistic for that summary mean change calculation was significant (ie, studies were not homogeneous).

that the absolute summary mean [SD] change decrease in weight for lower-carbohydrate diets was 3.6 (1.2) kg (95% CI, 1.2-6.0 kg) and for higher-carbohydrate diets was 2.1 (0.3) kg (95% CI, 1.6-2.7 kg). This overlap in 95% CIs suggests no difference in weight loss between the lower- and higher-carbohydrate diets.

To evaluate the weight loss demonstrated in the studies with the lowest-carbohydrate content, we calculated the summary mean [SD] change in weight loss found in the 13 diets* of these diets with 71 participants. In this group of studies, we found a summary mean (SD) change in weight of −1.2 (−2.3) kg (95% CI, −5.7 kg to 3.3 kg). The result of the Q statistic suggests homogeneity; however, we note that these studies vary with respect to study design, including studies that are not randomized and that do not include a comparison group. Thus, based on the data, it can be concluded that lowest-carbohydrate diets did not result in significantly greater weight loss than lower-carbohydrate diets.

When we consider the 22 diets† with the greatest mean weight loss (ie, mean weight loss of ≥10 kg), we found that they varied widely with respect to carbohydrate content (mean [range], 97 [10-271] g/d of carbohydrate) (data not shown). However, these diets restricted caloric intake (mean [range], 1077 [525-1800] kcal/d), were longer in duration (mean [range], 142 [42-365] days), and included participants who were significantly overweight at the start of the diets (mean [range], 101 [84-183] kg) (data not shown). These results suggest that these 3 variables may be more important predictors of weight loss than carbohydrate content.

Change in Serum Lipid Levels. For all studies and participants of lower-carbohydrate diets, no change was found in any of the serum lipid levels (ie, the 95% CIs for the summary mean [SD] change in total, LDL, and HDL cholesterol, and triglycerides levels all included 0) (Table 5). However, heterogeneity and paucity of studies complicate the interpretation of the outcomes of serum lipid levels. In contrast, among the more homogeneous group of studies of higher-carbohydrate diets, we found a significant decline in total cholesterol levels (summary mean [SD] change, −8.1 [1.4] mg/dL; 95% CI, −5.5 to −10.8 or −0.21 [0.04] mmol/L; 95% CI, −0.14 to −0.28 mmol/L) but not in the other serum lipid levels (95% CIs include 0).

From the 3 studies[80,106,121] of lowest-carbohydrate diets that reported data for total cholesterol levels for 36 participants, we found no change in serum lipid levels (summary mean [SD] change for total cholesterol, +0.1 [28.0] mg/dL; 95% CI, −54.8 to +55.1 mg/dL or 0.0026 [0.73] mmol/L; 95% CI, −1.4 to 1.4 mmol/L) (data not shown). None of the studies specifically evaluated the effect of lower-carbohydrate diets on serum lipid levels among participants with hyperlipidemia, and only 1 study[79] reported outcomes for serum lipid levels for participants with diabetes.

Change in Fasting Serum Glucose and Insulin Levels. No change was observed in either fasting serum glucose or insulin levels among recipients of either lower- or higher-carbohydrate diets—even among those participants with the greatest weight loss or those participants receiving the lowest-carbohydrate diets (Table 5). Only 1 small study[78] (9 participants) specifically evaluated the effect of lower-carbohydrate diets on fasting serum glucose or insulin levels among obese participants with diabetes (both 95% CIs include 0) (data not shown).

Change in Systolic Blood Pressure. We found no change in systolic blood pressure after diet in participants receiving either lower- or higher-carbohydrate diets. Four studies[39,42,95,102] of 173 recipients of lower-carbohydrate diets demonstrated a summary mean (SD) change in decrease in blood pressure of 0.7 (5.2) mm Hg (95% CI, +10.8 to −9.5 mm Hg) (Table 5).

Outcome Variables for Low-Carbohydrate Diets

To determine the effect of diet and participant characteristics on the outcomes of interest, a weighted analysis of variance was performed (TABLE 6). The weighted analysis of variance was used because the outcome variables are correlated; the diets vary with respect to total caloric content, duration, and carbohydrate content; and to avoid the use of a threshold to define what constitutes a lower-carbohydrate diet. Because only a few studies evaluated all of the dietary, participant, and outcome variables of interest, we were limited in our ability to include all studies or all variables in this analysis. The results of the analysis of variance using all diet data from all studies reporting weight loss, baseline weight, age, sex, and diet variables demonstrate that weight loss was significantly associated with longer diet duration ($P=.008$) and baseline weight ($P<.001$). For obese participants, restriction of calorie intake also was associated with weight loss, albeit not statistically significant after applying the Bonferroni adjustment ($P=.03$) (Table 6). Reduced carbohydrate content was not significantly associated with weight loss.

For all diets and all participants, reductions in LDL cholesterol levels were associated with high baseline weight ($P=.005$), weight loss ($P=.005$), younger age ($P=.004$), restriction of calorie intake ($P=.002$), and longer diet duration ($P=.002$)(Table 6). Overall dietary and participant characteristics were not significantly associated with changes in total cholesterol, HDL cholesterol, or triglyceride levels. Reductions in fasting serum glucose and insulin levels were consistently associated with longer diet duration ($P=.01$ and $P=.002$, respectively). Restriction of carbohydrate intake was not significantly associated with changes in serum lipid levels, change in fasting serum glucose levels, or systolic blood pressure.

COMMENT

Our quantitative synthesis of the 107 studies of 94 diets from the English-language literature on the efficacy and

*References 53, 65, 80, 82, 83, 106, 116-118.
†References 23, 24, 26, 40, 44, 45, 67, 73, 78, 79, 95-97.

Table 6. Results of Weighted Analysis of Variance to Determine the Effects of Diets and Participants on Outcome Variables for Lower-Carbohydrate Diets*

Outcome	No. of Diets†	R^2‡	*P* Values§							
			Baseline Weight, kg	% Male	Mean Age, y	Carbohydrates, g/d	Caloric Content, kcal/d	Diet Duration, d	Weight Loss, kg‖	Reduction of Fasting Glucose, mg/dL¶
Weight loss, kg										
All diets, all participants	35	0.69	<.001	.04	.02	.90	.50	.008	...	...
RCT and R-Cross studies only	15	0.94	<.001	.90	.30	.10	.50	.06	...	...
Healthy volunteers	12	0.57	...	...	...	.40	.90	.08	...	...
Obese participants	33	0.33	...	...	...	.90	.03	.002	...	...
Diabetic participants	12	0.60	...	...	...	.40	.02	.30	...	...
Reduction in total cholesterol, mg/dL										
All diets, all participants	25	0.31	...	.20	.30	.20	.50	.30	.90	...
RCT and R-Cross studies only	9	0.88	...	.80	.80	.20	.30	.70	.40	...
Healthy volunteers	9	0.59	...	...	...	.10	.30	.80	.80	...
Obese participants	12	0.21	...	...	...	.70	.90	.90	.50	...
Diabetic participants	29	0.12	...	...	...	.90	.09	.90	...	...
Reduction in LDL cholesterol, mg/dL										
All diets, all participants	10	1.00	.005	.02	.004	.10	.002	.002	.005	...
RCT and R-Cross studies only	13	0.83	.01	.10	.06	.20	.50	.07	...	...
Healthy volunteers	11	0.21	...	...	...	.20	.50	.80	...	...
Obese participants	8	0.97	.30	.20	.90	.30	.20	.90	...	...
Diabetic participants	15	0.55	.07	.30	.30	.90	.10	.60	...	...
Increase in HDL cholesterol, mg/dL										
All diets, all participants	9	0.94	...	.90	.50	.60	.70	.20	.30	...
RCT and R-Cross studies only	19	0.19	...	.20	.70	.60	.70	.60	...	...
Healthy volunteers	12	0.26	...	...	...	.90	.20	.70	...	...
Obese participants	13	0.73	...	...	...	.20	.01	.003	...	...
Diabetic participants	20	0.02	...	...	...	.60	.90	.90	...	...
Reduction in triglycerides, mg/dL										
All diets, all participants	8	0.99	...	...	...	.04	.05	.20	.09	.40
RCT and R-Cross studies only	9	0.41	...	...	...	.90	.80	.30	.60	...
Healthy volunteers	22	0.19	...	...	...	.10	.70	.90	...	...
Obese participants	12	0.51	...	...	...	.10	.20	.70	.30	...
Diabetic participants	20	0.11	...	...	...	.50	.90	.40	...	.30
Reduction in fasting serum glucose, mg/dL										
All diets, all participants	10	0.79	...	...	...	.90	.10	.01	.06	...
RCT and R-Cross studies only	31	0.56	...	...	...	.30	.10	<.001	...	...
Healthy volunteers	17	0.36	...	...	...	.10	.20	.20	...	...
Obese participants	20	0.09	...	...	...	.50	.80	.30	...	...
Diabetic participants	27	0.61	...	...	...	.90	.003	.001	...	...
Change in fasting serum insulin, μIU/L										
All diets, all participants	49	0.24	...	...	...	.10	.90	.002	...	...
RCT and R-Cross studies only	26	0.46	...	...	...	.10	.40	<.001	...	...
Healthy volunteers	12	0.55	...	...	...	.03	.20	.70	...	...
Obese participants	16	0.35	...	...	...	.80	.20	.20	...	...
Diabetic participants	18	0.51	...	...	...	.10	.50	.003	...	...
Change in systolic blood pressure, mm Hg										
All diets, all participants	10	0.56	...	...	...	.30	.20	.20	.40	...
RCT and R-Cross studies only	9	0.53	...	...	...	.80	.90	.20	.50	...
Healthy volunteers	...	...	...	...	...	...	...	...	...	...
Obese participants	16	0.21	...	...	...	.90	.50	.40	...	...
Diabetic participants	8	0.34	...	...	...	.80	.80	.50	...	...

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; RCT, randomized controlled trial; R-Cross, randomized crossover trial. Ellipses indicate insufficient data to calculate.

*See Table 4 for the conversion of conventional units to SI units.

†Number of diets refers to the number of dietary interventions that could be included in each analysis. To be included in the analysis of variance, a study had to report data for each of the predictor and outcome variables of interest. For example, studies of 10 dietary interventions provided data on change in fasting serum glucose levels and included information about the carbohydrate content of the diet, the caloric content of the diet duration, and participants' weight loss. However, the RCTs and R-Cross studies did not provide sufficient data about weight loss as a predictor of reduction in fasting serum glucose levels.

‡Using the weighted analysis of variance (weighted by the number of participants who finished each diet), the R^2 was calculated to estimate the amount of variance in the outcome variables accounted for by the predictor variables.

§*P* value associated with the effect of these predictor variables on the outcome variables.

‖Weight loss was included as a predictor variable for the weighted analyses of variance for change in serum lipid, fasting serum glucose, and fasting serum insulin levels, and systolic blood pressure as allowed by the availability of data.

¶Reduction of fasting serum glucose levels was included as a predictor variable for the weighted analyses of variance for change in serum triglyceride levels as allowed by the availability of data.

safety of low-carbohydrate diets suggests that there is insufficient evidence to make recommendations for or against the use of these diets. Despite the large number of Americans who have apparently adopted this approach to weight loss and/or weight maintenance, we know little of its effects or consequences. In particular, these diets have not been adequately evaluated for use longer than 90 days, for individuals aged 53 years or older, or for use by participants with hyperlipidemia, hypertension, or diabetes. The lowest-carbohydrate diets (eg, ≤20 g/d of carbohydrates, the recommended threshold for some of the most popular diets) have been studied in only 71 participants for whom no data on serum lipid, fasting serum glucose, and fasting serum insulin levels or blood pressure was reported.

We found insufficient evidence to conclude that lower-carbohydrate content is independently associated with greater weight loss compared with higher-carbohydrate content. We did find, however, that diets that restricted calorie intake and were longer in duration were associated with weight loss. Given the limited evidence in this review, when lower-carbohydrate diets result in weight loss, it also is likely due to the restriction of calorie intake and longer duration rather than carbohydrate intake. Lower-carbohydrate diets were not associated with adverse effects on serum lipid levels, fasting serum glucose levels, or blood pressure. However, because few studies reported on these outcomes, this systematic review lacked statistical power to detect small changes in these measures.

The heterogeneity of all the studies included in this review precludes drawing conclusions from the synthesis of the total group of studies. The statistically significant weight loss demonstrated when we compared all studies of lower- and higher-carbohydrate diets using the threshold of 60 g/d of carbohydrates was not confirmed by any other analyses (eg, evaluating the recipients of diets containing ≤20 g/d of carbohydrates or the participants with the greatest weight loss). We attribute this finding to the inclusion of studies of lower-carbohydrate diets with relatively short durations for obese participants in whom significant weight loss was achieved while using diets of 60 g/d or less of carbohydrates, primarily through restriction of calorie intake. It may be that these obese participants were better able to tolerate the restriction of calorie intake while using lower-carbohydrate diets than while using higher-carbohydrate diets. This observation suggests the need for additional studies of isocaloric diets with different carbohydrate contents in which participants are specifically assessed for symptoms of hunger and on the tolerability of the diet.

Our analyses were limited by a small number of studies that evaluated more than 1 of the outcomes of interest or that provided sufficiently detailed information about their participants or dietary intervention. Specifically, our systematic review highlights 5 significant gaps in the published literature of low-carbohydrate diets. First, the lack of adequate long-term follow-up data significantly limits our understanding of the efficacy and safety of low-carbohydrate diets. In particular, the long-term effects of low-carbohydrate diets on serum lipid, fasting serum glucose, and fasting serum insulin levels and blood pressure may differ between hypocaloric diets intended for weight loss and isocaloric diets intended for weight maintenance. Second, we were not able to evaluate the effects of these diets on different racial/ethnic groups. The absence of data regarding the efficacy and safety of lower-carbohydrate diets by race/ethnicity critically limits our ability to make participant-specific recommendations about these diets. Third, because exercise can have a significant effect on weight loss, we had hoped to include a measure of energy expenditure as a covariate in our analyses. We were unable to report data on exercise because many studies either did not report any information about participants' exercise patterns or simply stated that participants were encouraged to maintain baseline levels of exercise. Fourth, some of the included diets provided counseling or other supportive measures to encourage participants to adhere to the dietary intervention. The heterogeneity of the information reported about how adherence was measured limited our ability to include them in our analyses. Finally, many of the included studies reported only the number of participants who completed the dietary intervention. Among those studies that reported both the total number enrolled and the total number who completed the intervention, very few performed an intention-to-treat analysis. This limitation of both the lower- and higher-carbohydrate diets has the potential to bias the results in the direction of overstating the effects of the dietary intervention.

Our search strategies may have introduced biases into our results. First, we only included English-language studies. We found 17 foreign-language articles that we could not exclude on the basis of the English title or abstract. Extrapolating from our finding that 94 evaluated dietary interventions of 60 g/d or less of carbohydrates, it is likely that about a third of these would have evaluated lower-carbohydrate diets. However, we believe that the data from these estimated 5 or 6 foreign-language articles that may have met our inclusion criteria would not have changed the result of our analyses, as to do so all of these studies would need to have included significantly larger number of participants than the included studies, found very different results than those described, or evaluated diets for more than 90 days. Given the important cultural and ethnic differences in dietary habits, including foreign-language studies may have increased the heterogeneity of the participants evaluated. Second, our search was limited to MEDLINE and the bibliographies of retrieved publications. Although the major nutrition science publications are included in the MEDLINE database, we may have missed some relevant ar-

ticles. Given the multiple clinical outcomes evaluated, we did not perform a formal analysis of publication bias.

The results of our systematic review suggest that if participants without diabetes tolerate a lower-carbohydrate diet better than a higher-carbohydrate alternative, this diet may be an effective means of achieving short-term weight loss without significant adverse effects on serum lipid levels, glycemic control, or blood pressure. However, there is insufficient evidence to recommend or condemn the use of these diets among participants with diabetes or for long-term use. Because of the complex relationships between serum lipid levels, plasma insulin levels, cortisol and glucogon levels during dieting,[88] and because of the claim by some proponents of low-carbohydrate diets that these diets work best when producing ketosis,[6] future evaluations of lower-carbohydrate diets should enroll participants with and without diabetes and with and without abnormal lipid levels to more fully describe the effects of lower-carbohydrate (sometimes called "ketogenic") diets on lipid and glycemic indices and ketogenesis.

Despite the abundance of lay literature on the topic of low-carbohydrate diets, to date our study is the first published synthesis of the evidence from the English-language literature. Our results demonstrated the marked discordance between the knowledge needed to guide dietary choices and the information that is available in the medical literature. Investigations that will examine the long-term effects and consequences of low-carbohydrate diets among both older and younger participants with and without diabetes, hyperlipidemia, and hyperkalemia are in urgent need.

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EXHIBIT 2

Changes in Plasma Lipids During Renin–Angiotensin System Blockade by Combination Therapy (Enalapril Plus Valsartan) in Patients With Diabetes and Hypertension

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Abstract: There is experimental evidence of an interaction between the angiotensin system and lipid metabolism. The aim of this study was to evaluate whether a block of the angiotensin system achieved both by ACE inhibition and angiotensin II–AT1 receptor blockade could affect the plasma lipid profile and, if so, what relationship exists between these possible changes and glucose metabolism and blood pressure. In 50 patients with type 2 diabetes and hypertension, treated with diabetes drugs and enalapril, we evaluated the glycemic and lipid profile together with the HOMA insulin-resistance index, blood pressure and microalbuminuria at baseline and 3 months after the addition of valsartan. At the second evaluation, blood pressure was reduced as expected, whereas the glycemic profile, the HOMA index, and the body mass index were unchanged. Total cholesterol, LDL-c, and apoprotein B were reduced during combination therapy ($P = 0.003$, $P = 0.001$, and $P = 0.004$, respectively), plasma HDL-c was slightly though significantly increased ($P = 0.024$), whereas apoprotein A and triglyceride levels did not change. After adjustment for the insulin resistance index and for blood pressure, the reduction of LDL-c and apoprotein B and the increase in HDL-c remained significant. The variation in lipid profile was not related to the changes in blood pressure. Moreover, the addition of valsartan to enalapril was associated with a reduction in microalbuminuria, which remained significant after adjustment for LDL-c or blood pressure changes. Thus, the greater degree of renin–angiotensin system blockade or specific pharmacodynamic effects of valsartan could account for the changes in plasma lipid profile observed in this study.

Key Words: angiotensin, lipid metabolism, type 2 diabetes, valsartan, enalapril

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Atherogenesis is characterized by complex phenomena among which the renin–angiotensin system (RAS) is now recognized as an important modulating factor. The key role of low-density lipoproteins (LDL) and their modified forms in determining the vascular cell dysfunction and injury is well known.[1,2] Recently there has been increasing evidence of an interaction between hyperlipidemia and the RAS activation. LDL up-regulates AT1 receptor expression,[3,4] and, conversely, angiotensin II up-regulates specific endothelial LOX-1 receptors for ox-LDL through AT1-receptor activation and facilitates oxidation of LDL and its uptake by monocytes/macrophages (among the reported mechanisms involved in this process are an increment in cellular proteoglycan content and fucoidin-binding receptors and increased cellular CD36 mRNA expression).[5–10] Moreover, recent experimental evidence in hypertension has linked the modifications associated with the atherogenesis occurring in vascular walls and plasma with both the lipid profile and metabolism and the angiotensin system. The SHR animal model, which shows increased angiotensin II levels, renin activity, angiotensin receptors, and angiotensinogen mRNA, exhibits an up-regulation of the LOX-1 receptor for ox-LDL expression.[11,12] Moreover, LDL derived from hypertensive patients is more susceptible to lipid peroxidation than LDL derived from normotensive controls.[12] Diabetes mellitus magnifies the risk of cardiovascular damage and leads to accelerated atherosclerosis.[13] Various elements such as dyslipidemia, hyperglycemia, insulin resistance, and platelet activation and aggregation, together with the increased prevalence of hypertension, cooperate in the increase of cardiovascular morbility and mortality in patients with diabetes.[13] In addition, microalbuminuria has been shown to be an independent predictor of increased cardiovascular risk in diabetic patients.[14] The aim of this study was to evaluate in this high-risk group of patients with diabetes and hypertension whether a greater blockade of the RAS achieved by inhibiting the system both at the converting enzyme and at the AT1 receptor levels could affect the plasma lipid profile and, if so, what relationship exists between these possible changes and changes in glucose metabolism and blood pressure. As a secondary endpoint we investigated whether microalbuminuria, an index of microvascular damage, could be reduced by the addition of valsartan to enalapril and whether this possible change was accounted for by blood pressure or lipid profile variations.

METHODS

We studied 62 consecutive patients with both type 2 diabetes and hypertension[15] referring to our Hypertension

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Center with the following characteristics: all were treated with enalapril 10 mg/d for hypertension and with diet and/or antidiabetic medications for diabetes (4 patients with diet therapy, 11 with biguanides, 10 with sulfonylureas, 26 with biguanides and sulfonylureas, 8 with biguanides and sulfonylureas and acarbose, and 3 with insulin). Moreover, the patients were included in the study if clinical blood pressure was greater than 130/80 mm Hg despite the enalapril treatment and the clinical condition did not require a change in diet and/or antidiabetic medications.

In 50 patients (group 1) valsartan 80 mg/d was added to the drugs already taken (enalapril and antidiabetics). Twelve consecutive patients (control group: group 2) agreed to continue the antihypertensive treatment with enalapril alone for the following 3 months.

All the patients were evaluated twice (at baseline and after 3-month follow-up) with clinical visits including calculation of body-mass-index (BMI), measurements of sphygmomanometric blood pressure, evaluation of ambulatory blood pressure, and laboratory examinations. In addition, the 24-hour microalbuminuria was measured at both examinations.

In all subjects, blood sampling was obtained to determine the glycemic profile (fasting glucose, fasting insulin, Hb_{A1c}), total cholesterol, HDL-c, LDL-c, triglycerides, apoprotein A, apoprotein B. The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated according to the formula: HOMA-IR index = [fasting insulin (pmol) × fasting glucose (mmol/L)]/22.5.[16]

Blood Pressure Evaluations

Sphygmomanometric blood pressure was determined in the morning between 9:00 and 10:00, and systolic blood pressure, diastolic blood pressure, and pulse pressure (arithmetic difference between systolic and diastolic measures) were considered in subsequent analyses. Afterward a 24-hour blood pressure monitoring was performed. The subjects were previously instructed to take the antihypertensive treatment (enalapril 10 mg) at 7:00 AM, even on the days of the visits. Then the patients were instructed to provide 24-hour urines for microalbuminuria detection and were submitted to a fasting blood sampling. The blood pressure monitoring was performed with a Takeda 2430 (A&D Co) monitor set to take a measurement every 15 minutes. The 24-hour systolic and diastolic blood pressures were evaluated. After 3 months all the subjects again underwent a sphygmomanometric evaluation of blood pressure, 24-hour ambulatory blood pressure monitoring, and blood sampling. Microalbuminuria was also measured at 3-month follow-up. All subjects took medications regularly, taking antihypertensive drugs at 7:00 AM even on the day of the test (50 patients, enalapril 10 mg plus valsartan 80 mg; 12 patients, enalapril 10 mg).

Statistical Analysis

Data are presented ± SD, unless skewed when median and interquartile range (IQR) were reported. A χ^2 test was used to compare the prevalence of the various treatments for diabetes (diet, oral antidiabetic treatment, insulin) in the 2 groups of patients (group 1, patients in whom antihypertensive treatment was increased to enalapril plus valsartan after the first evaluation; group 2, patients who stayed on enalapril alone).

The Mann-Whitney *U* test was performed to compare hemodynamic and metabolic parameters between group 1 and group 2. A general linear regression model was fitted to the data to assess changes over time of blood pressure, lipid profile, and (log-transformed) microalbuminuria, with calculation of Huber-White robust standard errors to account for intrapatient correlation in time. The observed changes were also controlled for insulin resistance and blood pressure or lipid profile in a multivariate model. Pearson correlation coefficient was computed to measure the association between changes in blood pressure and in lipid profile. Stata 7 (StataCorp, College Station, TX) was used for computation. A 2-sided P value <0.05 was retained for statistical significance.

RESULTS

In 50 type 2 diabetic patients whose antihypertensive treatment was increased after the first evaluation adding valsartan to enalapril (group 1), the lipid profile was significantly changed after a 3-month follow-up. During combination therapy, total cholesterol, LDL-c, and apoprotein B levels were reduced by 16 mg/dL, 14 mg/dL, and 10 mg/dL, respectively, when compared with the values observed at the first evaluation, whereas the plasma HDL-c was slightly though significantly increased by 2 mg/dL (Table 1). Figure 1 shows the individual changes in LDL-c and in HDL-c. The apoprotein A and triglyceride levels did not significantly differ from the values found at the first evaluation (Table 1).

In these group 1 patients, the glycemic profile and the insulin-resistance HOMA-IR index at the follow-up examination were similar to those found at the first evaluation, but the sphygmomanometric blood pressure as well as the 24-hour ambulatory blood pressure were significantly reduced as expected by the addition of valsartan to enalapril (Table 1). Moreover, the body-mass index did not change after the 3-month follow-up (first evaluation, group 1, 26.8 ± 4.8 kg/m^2; second evaluation, group 1, 26.5 ± 4.6 kg/m^2).

At baseline (enalapril monotherapy in both groups), the lipid profile, the glycemic profile, and the HOMA-IR index were similar between group 1 and group 2 (12 patients who stayed on enalapril alone in the following 3 months) (Table 1). Moreover, the body-mass index was similar between the groups (group 2, 26.9 ± 4.8 kg/m^2), and the prevalence of dietary treatment and/or oral antidiabetic drugs and/or insulin treatment was similar in the 2 groups of patients. The clinical blood pressure, pulse pressure, and metabolic profile in group 1 did not differ from those in group 2 (Table 1). These patients did not show any statistically significant change in lipid and glycemic profile, HOMA-IR index, or body-mass index during the follow-up period (data not shown).

In group 1 patients who showed an improvement in lipid profile after combination therapy, after adjustment for insulin resistance index and for blood pressure, the reduction of LDL-c and apoprotein B and the increase in HDL-c remained significant and did not change in magnitude (adjusted differences: reduction of 13.6 mg/dL, reduction of 10.4 mg/dL, and increase of 3.9 mg/dL, respectively). The variation in lipid profile

TABLE 1. Lipid Profile, Glycemic and Metabolic Parameters, and Blood Pressure Values in Group 1 (Patients Who Added Valsartan to Enalapril During the 3-Month Follow-up) and in Group 2 Patients (Subjects Who Remained on Enalapril Alone)

	Group 1			Group 2
	Baseline* (Enalapril)	Follow-up (Enalapril + Valsartan)	P	Baseline* (Enalapril)
Lipid profile				
Total cholesterol (mg/dL)	220.9 ± 44.9	205.5 ± 35	0.003	224 ± 42
LDL-c (mg/dL)	134.6 ± 36	121 ± 31.1	0.001	137.4 ± 29
HDL-c (mg/dL)	50.5 ± 14.9	52.5 ± 15.3	0.024	51.12 ± 12
Apoprotein B (mg/dL)	114 ± 31	103.8 ± 22	0.004	117 ± 34
Apoprotein A (mg/dL)	139.1 ± 26.3	133.3 ± 28.5	ns	140.2 ± 30
Tryglicerides (mg/dL)	178.8 ± 109.7	160.5 ± 84.6	ns	172.52 ± 97
Glycemic and metabolic parameters				
Fasting glucose (mg/dL)	170 ± 45	160 ± 47	ns	165 ± 48
Fasting insulin (μU/mL)	13.1 ± 9.8	14 ± 16.6	ns	12.5 ± 11.3
HOMA-IR index	5.29 ± 4.25	5.49 ± 4.65	ns	5.05 ± 4.36
Blood pressure values				
Sphygmomanometric SBP (mm Hg)	151 ± 20	136 ± 23	<0.001	153 ± 20
Sphygmomanometric DBP (mm Hg)	84 ± 10	76 ± 13	0.002	84 ± 12
Sphygmomanometric PP (mm Hg)	67 ± 15	60 ± 16	<0.001	68 ± 16
24-hour SBP (mm Hg)	145 ± 15	139 ± 14	0.01	146 ± 17
24-hour DBP (mm Hg)	78 ± 7	75 ± 6	0.05	77 ± 8

*$P > 0.01$ comparing baseline values between groups 1 and 2 (Mann-Whitney u test).
SBP indicates systolic blood pressure; DBP indicates diastolic blood pressure; PP indicates pulse pressure; LDL-c indicates low-density lipoprotein-cholesterol; HDL indicates high-density lipoprotein-cholesterol.

was not related to the changes in blood pressure as indicated by the lack of relationship between the variations in LDL-c and systolic, diastolic and pulse pressure changes occurring after the 3-month follow-up (Pearson R ≤ 0.27, ns, in all cases).

Moreover, the addition of valsartan to enalapril was associated with a reduction of microalbuminuria [median (IQR) 5.9 (2.4–12) at first evaluation and 1.0 (0.6–2.9) at second evaluation, $P < 0.001$]. The changes observed in (log-transformed) microalbuminuria remained significant and of the same magnitude after adjustment for LDL or blood pressure changes.

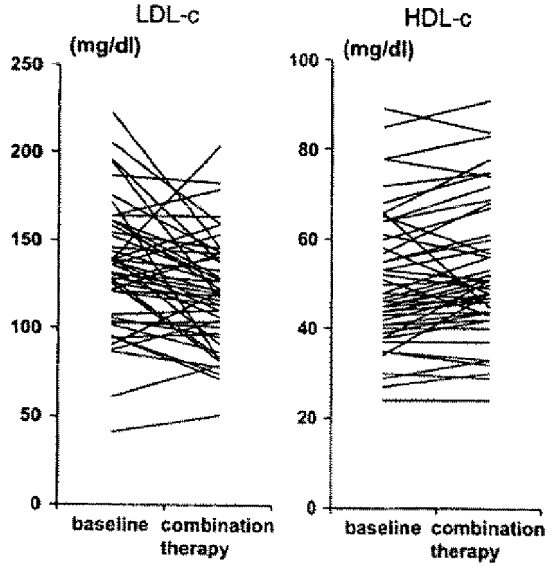

FIGURE 1. Changes in LDL-cholesterol ($P = 0.001$) and HDL-cholesterol ($P = 0.024$): baseline (enalapril) to combination therapy (enalapril plus valsartan).

DISCUSSION

Several experimental studies have suggested an interaction between dyslipidemia and the angiotensin system.[3–12] In rabbits a marked increase of AT1 receptor density has been shown in hypercholesterolemia.[4] In humans a close relationship has been found between AT1 receptor density and plasma LDL-cholesterol and the use of statins to lower cholesterol was associated with AT1 receptor down-regulation.[17] In this study we report in type 2 diabetic patients with hypertension that the greater inhibition of the RAS obtained with ACE inhibitors plus AT1 receptor blockade was associated with a significant reduction of LDL-cholesterol together with a reduction of apoprotein B and a slight though significant increase in HDL-c as well as the expected blood pressure lowering induced by the drugs.

Some reports on animals and in man have shown a reduction in LDL plasma levels or in cholesterol synthesis with RAS inhibition.[18–20] However, other studies have failed to find a lipid profile modification during ACE-inhibitor treatment.[17,21–24]

In humans, recently, a decrease in total cholesterol and LDL-cholesterol was seen in 60 hypertensive patients after a 12-week treatment with 80 mg daily valsartan, whereas no change in apoprotein B lipoprotein was observed.[25] Previously, significant decreases in total cholesterol, apolipoprotein B,

and apolipoprotein A have been seen during therapy with valsartan when compared with captopril in patients with type 2 diabetes mellitus and nephropathy.[26]

In our study, the improved lipid profile may be a result of the more complete block of the RAS obtained by using both enalapril and valsartan. Moreover, in the control group of patients who did not receive valsartan added to enalapril, no change was observed in lipid plasma levels.

Although a close interaction between the glucose and lipid metabolisms is well established, in our study glucose metabolism was unchanged in the patients who added valsartan to the previous treatment with enalapril, as indicated by similar Hb_{A1c} levels and glucose and insulin values. Moreover, similar body mass indices were found in these patients at the 2 evaluations. ACE inhibitors but not angiotensin II receptor antagonists seem to be associated with an improvement of insulin resistance[13,27]; in this study of diabetic hypertensives the HOMA-IR insulin resistance index was unchanged after the 3 months following the additional treatment with valsartan. In addition, changes in lipid profile were shown to be independent of insulin resistance and blood pressure in the multivariate model. Thus, it is not likely that insulin sensitivity could have accounted for the beneficial effects on the lipid profile seen in our study. However, it has to be acknowledged that we did not investigate insulin sensitivity with more sensitive methods.

Although the relative roles of AT1 and AT2 receptors in vascular remodeling are not completely clarified, most of the known vascular effects of angiotensin II are mediated through AT1 receptors, and their blockade has shown beneficial effects on atherogenesis. There is experimental evidence for an involvement of AT1 receptors in the cross-talk between lipid metabolism and angiotensin.[28–32] The lecitin-like receptor LOX-1 for ox-LDL is thought to participate actively in atherogenesis, and AT1-receptor blockade by losartan has been shown to decrease the enhanced LOX-1 expression up-regulated by both angiotensin II and high-cholesterol diet in vitro and in vivo.[6,31] Moreover, angiotensin II increases macrophage-mediated modification of LDL via a lipoxygenase-dependent pathway through AT1 receptors, and valsartan (but not AT2 receptor blockade) was shown to inhibit the lipoxygenase activity and, subsequently, the ability of the cells to modify LDL and the measured monocyte chemiotaxis.[32] Thus, in this study, the addition of valsartan to enalapril may have affected the lipid profile through the AT1 blockade. Moreover, in this study, the reduction of the renal damage expressed by microalbuminuria observed with valsartan remained significant after adjusting for blood pressure or LDL-c levels, possibly indicating a specific protective effect against microvascular injury as a result of using this drug, as already suggested by others.[33] Alternatively, a more prolonged follow-up may be needed to detect a potential relationship between renal protection and lipid metabolism changes.

Although not much is known about the local concentration of angiotensin II within the vascular wall, it has been suggested that locally generated angiotensin II may have autocrine and paracrine effects. The importance of tissue ACE activity in the interaction between the angiotensin system and lipid metabolism is shown by the relative difficulty in inhibiting tissue ACE activity compared with serum ACE levels, as a higher dose of ACE inhibitor is needed in vivo for the inhibition of neointimal formation.[34] The changes observed in the lipid profile could be ascribed, at least in part, to the action of valsartan at the vascular wall level.

CONCLUSION

The modulation of the lipid metabolism observed in our study may depend on the greater degree of block of the RAS obtained by combination therapy or on a specific pharmacodynamic effect of valsartan.

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EXHIBIT 3

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Effects of continuous combined conjugated estrogen/medroxyprogesterone acetate and 17β-estadiol/norethisterone acetate on lipids and lipoproteins

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Abstract

Objectives: Various estrogen/progestogen combinations used in hormonal replacement therapy (HRT) have been reported to influence lipid and lipoprotein fractions differently. This motivated a comparative study where the two continuous combined regimens most commonly used in Sweden during the 1990s have been studied regarding effects on lipid profile. *Methods:* In a 1-year prospective, double-blind study, 62 post-menopausal women were randomized to conjugated estrogen (CE), 0.625 mg, and medroxyprogesterone acetate (MPA), 5 mg, or 17β-estradiol (E2), 2 mg, and norethisterone acetate (NETA), 1 mg. Serum concentrations of lipids and lipoproteins were measured at baseline and after 1 year of treatment. *Results:* Both treatment groups significantly lowered the lipoprotein(a) (Lp(a)) levels. The CE/MPA group showed no significant changes in total cholesterol (TC), high-density (HDL) and low-density lipoprotein (LDL), but a significant increase of triglyceride (TG) levels. The E2/NETA group developed a significant lowering of total cholesterol, HDL, and LDL, but no significant changes of TG levels. The magnitude of change in serum concentrations of total cholesterol, HDL and TG differed significantly between the two treatment groups. *Conclusions:* Continuous combined treatment with CE/MPA and E2/NETA equally lowered Lp(a), an important risk factor for cardiovascular disease in women. Apart from this, the two treatments produced different effects on lipids and lipoproteins, findings that are more delicate to interpret.

Keywords: Medroxyprogesterone acetate; Norethisterone acetate; Lipoprotein(a)

1. Introduction

Estrogens are known to induce beneficial effects on blood lipids and lipoproteins. In theory, this has been looked upon as factors contributing to a reduced risk for arteriosclerosis in post-menopausal women.

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However, if hormonal replacement therapy (HRT) actually protects women from cardiac death is still a matter of debate. In addition, the overall effect of a combination with estrogen and progestogen still needs to be elucidated.

In earlier studies of lipid metabolism, it has been shown that both the dosage and the type of progestogen are of importance for the lipoprotein, cholesterol and triglyceride (TG) fractions. More androgenic progestogens like norethisterone acetate (NETA) from the 19-nortestosterone group may be more prone to increase cholesterol fractions than derivates of 17-hydroxyprogesterone, e.g. medroxyprogesterone acetate (MPA) [1–3]. Conversely, the less androgenic MPA may not counteract the estrogen influence on triglycerides [4]. Studies directly comparing combinations with MPA and NETA have, to our knowledge, not been done before. As continuous combined HRT is now the most frequently prescribed HRT it was of obvious interest to accomplish a comparative study of the two continuous combined HRT's most commonly used in Sweden during the 1990s. Earlier, lipid changes have been investigated in trials with the two types of HRT separately, and some of the results are described below.

A study by Munk-Jensen et al. [5], showed some decrease of high-density lipoprotein (HDL) with a continuous combination of 17β-estradiol and norethisterone acetate (E2/NETA). Furthermore, Ylikorkala et al. [6] have shown that E2 in combination with NETA decreased HDL. One study showed almost no counteracting effects of MPA on serum lipid changes induced by conjugated estrogen (CE) alone [7]. In a study by Lobo et al. [7], CE and MPA increased HDL and TG and decreased total cholesterol (TC), low-density lipoprotein (LDL) and serum lipoprotein(a) (Lp(a)) [7]. Finally, comparisons with studies using sequential HRT are hazardous since duration as well as doses of the progestogens used are different. The effects of different estrogens have been reported in a meta analysis by Godsland. CE and E2 produced similar effects on blood lipids and lipoproteins [4].

The purpose of the present study was to compare the effects of a continuous treatment with 0.625 mg conjugated estrogen and 5 mg medroxyprogesterone acetate to 2 mg 17β-estradiol and 1 mg norethisterone acetate on blood lipid and lipoprotein profiles in post-menopausal women.

2. Material and methods

2.1. Study population

The study was carried out in five gynecological centers in Sweden between March 1997 and January 1999. Sixty-six healthy women with climacteric symptoms were recruited through advertisement or in connection with clinical visits. The inclusion criteria were: healthy women with an intact uterus, aged 52 years or more and being at least 2 years post-menopausal (women who had never used HRT). To include current users, the requirement was that HRT should have been going on for at least 2 years. However, for those women a washout period of at least 2 months before study start was carried out. The exclusion criteria were: adenomatous hyperplasia with or without atypia, undiagnosed vaginal bleeding, history of cancer of any kind, active liver disease, cardiovascular or thromboembolic disease, uncontrolled hypertension, diabetes and chronic medication with barbiturates and antiepileptic or psychiatric drugs such as antidepressants or bensodiazepins. In addition, smokers and women with a body mass index (BMI) above 31 kg/m^2 were excluded (three women). However, lipid and lipoprotein values outside the normal range at screening did not constitute an exclusion criterion. Finally, 62 women were included in the study. The use of lipid lowering agents, statines, or steroid hormones other than study medication was not permitted during the study period.

At the screening visit, none of the women had taken any drug liable to interfere with lipoprotein metabolism (statines or diuretics) in the 3 months preceding this visit. All the women had normal renal and hepatic function before and during treatment as indicated by routine biochemical tests. In total, three women used anti-hypertension drugs (verapamil, felodipin and atenolol in combinations with enalapril) during the study. Before taking part (screening visit), the women underwent a physical examination, including breast and gynecological examinations. A transvaginal ultrasonography for the determination of endometrial thickness (double-layer technique) and an endometrial biopsy (the results are presented elsewhere) was performed. In case the endometrium was more than 10 mm, the women were not allowed in the study regardless of biopsy result. In addition, blood

pressure, weight and height were measured. Cervical smear and mammography were performed in case results were not available for the year prior to study onset. Blood samples for measuring plasma lipids and lipoproteins, kidney, liver and thyroid function were taken.

2.2. Study procedure

The effect of two different continuous combined regimens of HRT on blood lipids was evaluated in a 1-year prospective, double-blind randomized parallel-group design. The women were daily treated with either conjugated estrogen 0.625 mg and medroxyprogesterone acetate 5 mg or 17β-estradiol 2 mg and norethisterone acetate 1 mg. Enrollment and randomization were done at the baseline visit. A randomization list in blocks of four was computer generated by a statistician and kit numbers were assigned in ascending order at each investigative visit. In order to keep the medication blinded, a double-dummy technique with dark coated blisters was used. Apoteksbolaget AB, Stockholm, Sweden, performed the randomization and blinding. Novo Nordisk, Denmark, provided the study medication E2/NETA. Pharma-Vinci Medical Production, Frederiksvaerk, Denmark, produced the placebo tablets for E2/NETA. The CE/MPA combination and the corresponding placebo tablets were provided by Wyeth-Ayerst, Philadelphia, USA. Counting the study supplies at each visit assessed compliance.

Adverse events and concomitant medication were documented at each follow-up visit (at 2, 6 and 12 months). Reasons for early discontinuation of the study medication were documented by the investigator in the clinical report form by checking one of the following predefined categories: adverse reaction, other medical event, failed to return, unsatisfactory response—efficacy, protocol violation, other non medical event and patient request unrelated to study. At the last visit (12 months) the clinical examination was repeated, including pelvic and breast examination, transvaginal ultrasonography, endometrial biopsy and blood pressure. Again, a mammography was made. Finally, a blood sample was taken for measuring plasma lipid and lipoprotein, and thyroid function.

The primary outcome measure of this study was changes in blood lipid and lipoprotein patterns during long-term treatment with two different continuous combined HRT's.

The study was conducted and monitored according to Nordic Guidelines for Good Clinical Trial Practice (GCP). In addition, Standard Operation Procedures (SOP), defined by the sponsor, was used. The research protocol was approved by the Ethics committee of each center involved and by the Swedish Medical Product Agency following the rules of the Revised Declaration of Helsinki (Hong Kong, 1989).

2.3. Lipid assays

Blood samples from the antecubital vein were obtained under fasting conditions (minimum 12 h overnight) to determine plasma lipid and lipoprotein levels. From the same samples kidney, liver and thyroid functions were assessed. Within a half to one hour, the blood was centrifuged at 3000 revolutions per minute for 10 min. The fresh serum samples were analyzed consecutively within 24 h at one central laboratory, Nova Medical CALAB Clinical Trials Central, St. Görans Hospital, Stockholm, Sweden, and the laboratory procedures fulfilled all criteria for Good Laboratory Practice. Laboratory personnel were blinded to treatment regimen. Normal values from CALAB are presented in Table 2.

Levels of total cholesterol were measured by an enzymatic photometric test using cholesterol oxidase and peroxidase [8,9], and the interassay coefficient of variation was 1.9%. Triglyceride levels were assayed by a colorimetric enzymatic test using glycerol-3-phosphateoxidase [10], and the coefficient of variation was 3.5%. Concentrations of HDL cholesterol was determined by a direct enzymatic method [11] and the concentration of LDL cholesterol was calculated according to the method of Friedewald et al. [12] with an interassay coefficient of variation of 4.7%. Lp(a) concentrations were measured by an immunoturbidimetric analysis [13] with an interassay coefficient of variation of 8.8%.

2.4. Statistical methods

Conventional descriptive statistics are presented. When normality of data could not be rejected according to a Kolmogorov–Smirnov test, one-sample paired and two independent samples t-tests were used to analyze the within patient group changes and between

groups comparisons. When a Kolmogorov–Smirnov test yielded statistical significance (indicating non-normality of data) Wilcoxon type non-parametric tests were used. In transition matrices (four field tables), the McNemar test was used for analysis of changes within groups of lipid values from normal to above normal values (or vice versa). If the expected frequency of changes was too small (<5), a binomial test was used [14]. Exploratory, multivariate stepwise backward linear regression analyses have been performed on changes of lipids. The predictors were as follows: baseline lipid levels, age, BMI, diastolic and systolic blood pressure, height, weight, years from last menstruation, and the indicator variables (0/1), treatment.

The mass-significance problem has been assessed using Eklund's rule [15]. The standard statistical computer program Statistical Package for the Social Sciences (SPSS), version 11.0 was used for data handling and analyses. A P-value <0.05 was considered significant.

3. Results

3.1. Baseline characteristics

A total of 62 women were included in the study and the number of women included at the five centers was between 7 and 26. Of these, 47 completed the study ("completers") and 15 dropped out ("dropouts"), 6 mainly due to bleeding problems. Other reasons were headache ($n = 2$), abdominal pain ($n = 2$), afraid to use HRT ($n = 1$), constipation/breast tenderness ($n = 1$), depression ($n = 1$), upper respiratory infection ($n = 1$) and palpitations ($n = 1$). Between completers and dropouts there were no statistically significant differences in demographic data (Table 1) or baseline lipids tests. However, dropouts had significantly higher TSH concentrations compared to completers (2.4±0.33 mU/l versus 1.7±0.16 mU/l, $P < 0.05$).

When comparing the two treatment groups, there were no statistically significant differences of mean baseline characteristics what so ever (Table 1). Twenty-seven women (57.4%) had not been using HRT prior to the study.

3.2. Laboratory tests of thyroid function

Baseline values for thyroid function did not differ between the treatment groups (Table 2). After 1 year, the mean TSH levels were higher in the E2/NETA group than in the CE/MPA group (2.03 ± 0.19 mU/l versus 1.31 ± 0.19 mU/L; $P < 0.001$), however, well below the upper limit for normal values. Serum TSH increased in the E2/NETA group and decreased in the CE/MPA group (0.19 ± 0.131 mU/l versus −0.22 ± 0.154 mU/l (Table 2), although, the difference of change was not significant. At baseline, one woman in the CE/MPA group had a TSH value 48% above the normal reference range. This normalized after 1 year of treatment.

3.3. Changes in blood lipid levels and comparisons between treatment groups

According to tests of normality, data on changes of triglycerides, LDL, and Lp(a) concentrations deviated significantly from normality. Baseline lipid levels did

Table 1
Baseline characteristics (mean ± S.E.M.) of women treated with conjugated estrogen and medroxyprogesterone acetate (CE/MPA) or 17β-estradiol and norethisterone acetate (E2/NETA) and who completed the whole study, and the women who dropped out from the study

	CE/MPA ($n = 23$)	E2/NETA ($n = 24$)	Drop outs ($n = 15$)
Age (years)	55.8 ± 0.6	55.1 ± 0.6	57.5 ± 1.1
Time since menopause (years)	4.4 ± 0.5	4.7 ± 0.7	5.4 ± 0.9
Parity (n)	2.0 ± 0.2	1.6 ± 0.3	2.1 ± 0.3
Systolic blood pressure (mm Hg)	131 ± 3	134 ± 3	136 ± 3
Diastolic blood pressure (mm Hg)	75 ± 2	79 ± 2	79 ± 2
BMI (kg/m^2)	25.3 ± 0.5	25.8 ± 0.7	25.7 ± 0.9
Endometrial thickness (mm)	3.3 ± 0.4	2.9 ± 0.2	3.2 ± 0.4

Table 2
Lipid, and thyroid values (mean ± S.E.M.) at baseline (B) and at final visit (F) for women treated with conjugated estrogen and medroxyprogesterone acetate (CE/MPA) or 17β-estradiol and norethisterone acetate (E2/NETA). Normal ranges defined by the laboratory used are shown

	Visit	Normal range	CE/MPA ($n = 23$)	E2/NETA ($n = 24$)
			Mean ± S.E.M.	Mean ± S.E.M.
S-Cholesterol (mmol/l)	B	<6.5	6.1 ± 0.18	6.5 ± 0.23
S-Cholesterol (mmol/l)	F		6.1 ± 0.21	5.9 ± 0.17
S-Triglycerides (mmol/l)	B	0.6–2.2	1.1 ± 0.10	1.2 ± 0.13
S-Triglycerides (mmol/)	F		1.3 ± 0.13	1.1 ± 0.07
S-Lp(a) (g/l)	B	<0.30	0.20 ± 0.04	0.30 ± 0.06
S-Lp(a) (g/l)	F		0.13 ± 0.03	0.20 ± 0.04
S-HDL (mmol/l)	B	≥1.15	1.7 ± 0.09	1.8 ± 0.07
S-HDL (mmol/l)	F		1.7 ± 0.07	1.6 ± 0.06
S-LDL (mmol/l)	B	<5.0	3.9 ± 0.18	4.1 ± 0.25
S-LDL (mmol/l)	F		3.8 ± 0.20	3.6 ± 0.19
S-T4, free (pmol/l)	B	12–25	16 ± 0.40	16 ± 0.39
S-T4, free (pmol/l)	F		16 ± 0.39	16 ± 0.32
S-TSH (mU/l)	B	0.20–5.00	1.6 ± 0.29	1.8 ± 0.16
S-TSH (mU/l)	F		1.3 ± 0.19	2.0 ± 0.19

not differ between the treatment groups (Table 2). The changes in lipid and lipoprotein profiles during 1 year of treatment are shown in Fig. 1.

Total cholesterol did not change significantly in the CE/MPA treatment group (0.01 ± 0.14 mmol/l), whereas within the E2/NETA group TC decreased significantly (−0.64 ± 0.15 mmol/l, $P < 0.001$). The difference in change between groups was significant ($P < 0.005$).

Mean levels of HDL did not change significantly within the CE/MPA group, whereas a significant decrease ($P < 0.001$) was seen in the E2/NETA group. The difference in change between groups was significant (−0.01 ± 0.04 mmol/l versus −0.18 ± 0.04 mmol/l, $P < 0.01$).

LDL was significantly lowered within the E2/NETA group ($P < 0.025$) whereas no significant change could be seen in the CE/MPA group. When the change in the whole study group was analyzed together there was a significant decrease in LDL levels after 1 year of HRT (−0.31 ± 0.15 mmol/l, $P < 0.05$).

There was a significant increase of TG within the CE/MPA group ($P < 0.001$), whereas in the E2/NETA group no significant change was seen. The difference in change between the two treatment groups was significant (0.27 ± 0.07 mmol/l versus −0.12 ± 0.11 mmol/l, $P < 0.005$).

Compared to baseline, there was a significant decrease in Lp(a) levels in both treatment groups (CE/MPA: −0.07 ± 0.02 g/l, $P < 0.005$ versus E2/NETA: −0.10 ± 0.04 g/l, $P < 0.001$). However, the difference in change between the two treatment groups was not significant (Fig. 1).

Fig. 1 presents the P-values from 15 significance tests of which eight were significant on at least the 5% level. According to Eklund's rule [15] the results presented are significant on at least a mass-significance level of 2%. The analyses have been performed and results are presented according to the Per-Protocol (PP) principle due to the fact that complementary analyses according to the Intention-To-Treat principle (ITT, using last observation carried forward) did not significantly change the results found.

3.4. Multivariate regression analyses

Treatment and a number of possible predictors for change in blood lipids were used (see Statistical methods). The baseline lipid levels were negatively correlated to the change of all lipids in the regression

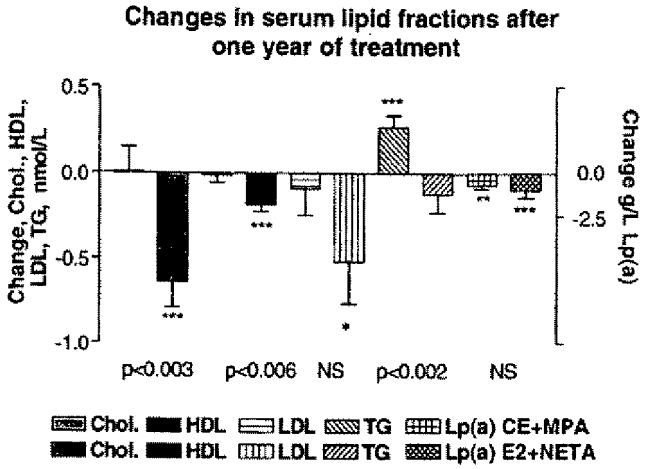

Fig. 1. Mean ± S.E.M. for changes from baseline of serum lipid and lipoprotein fractions after 1 year of treatment. Chol: total cholesterol (mmol/l); HDL: high-density lipoprotein (mmol/l); LDL: low-density lipoprotein (mmol/l); TG: triglycerides (mmol/l); and Lp(a): lipoprotein(a) (g/l). In the pair of bars, the left bars indicate daily treatment with 0.625 mg conjugated estrogen and 5 mg medroxyprogesterone acetate, (CE/MPA; $n = 23$), and the right bars treatment with 2 mg 17β-estradiol and 1 mg norethisterone acetate (E2/NETA; $n = 24$). P-values at the bottom of the graph show differences of change between the treatment groups. Significant changes from baseline within the groups are indicated $^{*}P < 0.05$, $^{**}P < 0.005$, $^{***}P < 0.001$, at each bar.

analyses ($P < 0.001$ in all tests) indicating that high baseline levels yielded a greater decrease in lipids than low baseline levels. Therefore, the multiple linear regression analyses were performed using change of concentration between baseline value and 1-year value (delta value = final value − baseline value). Treatment was a significant predictor for the change of TC ($P < 0.005$), HDL ($P < 0.005$), and TG ($P < 0.005$) concentrations. After adjustment for the baseline lipid levels and other predictors, the decrease in TC, HDL and TG levels in the E2/NETA group was significantly larger than in the CE/MPA group.

Age was positively correlated to change in TG levels ($P < 0.01$), indicating that older women, compared to younger, had a more pronounced increase in TG levels during the study (Table 3).

Table 3
Results from backward stepwise linear regression analyses on changes of lipid and lipoprotein concentrations after 1 year of treatment

Dependent variable	Predictors	Coefficient ± S.E.M.	t	$P<$	R^2
Delta TC	Treatment (0/1)	−0.56 ± 0.184	−3.05	0.005	
	BL, Height	−0.03 ± 0.017	−1.98	0.05	
	BL, TC (mmol/l)	−0.34 ± 0.090	−3.80	0.001	0.42
Delta HDL	Treatment (0/1)	−0.15 ± 0.050	−2.97	0.005	
	BL, HDL (mmol/l)	−0.29 ± 0.068	−4.27	0.001	0.40
Delta LDL	BL, LDL (mmol/l)	−0.59 ± 0.118	−5.02	0.001	0.36
Delta TG	Treatment (0/1)	−0.31 ± 0.104	−2.97	0.005	
	BL, Age	0.05 ± 0.018	2.83	0.01	
	BL, TG (mmol/l)	−0.47 ± 0.093	−5.09	0.001	0.52
Delta Lp(a)	BL, Lp(a) (g/l)	−0.52 ± 0.068	−7.67	0.001	0.57

The delta values are computed as final value minus baseline value (BL). TC: Total cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein; TG: triglycerides; and Lp(a): lipoprotein(a). Treatment is coded 0: CE/MPA and 1: E2/NETA. Coefficient: partial regression coefficients with standard error; S.E.M.: standard error; t: Student's t; R^2: coefficient of multiple determination.

3.5. Transition of lipid values

The transition of serum values in and out of the normal range was investigated for all lipid parameters. Only total serum cholesterol showed a significant difference in transition frequency between the treatments. In the CE/MPA group at baseline 16 women were within normal values and 7 above. After 1 year of treatment, 15 women were within normal and 8 above, of which 3 came from the normal baseline group and 5 were still above the normal baseline. Two women had their values normalized. At baseline, in the E2/NETA group, 13 women were within the normal range and 11 above. After 1 year, 19 women were normal and 5 above, whereas 6 women had shifted from increased levels to normal. The change in total cholesterol was significant within the E2/NETA group ($P < 0.05$). During the whole study period, one woman reported a serious adverse event: surgery behind the nose/conchotomized bilaterally. No case of thrombosis was reported.

4. Discussion

The major finding of the present study was that after 1 year of HRT significant differences in total cholesterol, HDL and triglycerides were found in women treated with conjugated estrogens and MPA compared to 17β-estradiol and NETA. However, a more significant finding was that both treatments effectively reduced Lp(a), which is regarded as an important risk factor for coronary heart disease (CHD) in women. As a matter of fact, the effects of HRT on coronary heart disease in post-menopausal women have, during the last decade, been a matter of debate. Epidemiological data suggest that HRT is associated with a reduction of CHD risk [16]. On the other hand, a significantly increased risk was noted in a recently published large, randomized, placebo controlled trial [17]. One reason for a negative impact on CHD risk might, however, be that other adverse haemostatic or vascular effects of HRT, such as the increased risk for thromboembolism overshadows the beneficial effects obtained by a change in lipid and lipoprotein levels. In addition, secondary prevention of myocardial infarction with HRT has not been successful either [18].

Albeit clinical data are inconsistent at the moment, results concerning individual risk factors are still of interest. In the present study, there was no change in HDL values after 1 year compared to baseline in the CE/MPA group. In contrast, HDL decreased in the E2/NETA group after one year of treatment, and this finding is at odds with previous studies [5,19]. Low levels of HDL-cholesterol in combination with high levels of TG seems to be a predictor for cardiovascular disease in women over 50 years of age [20,21]. In the present study, however, no women displayed low HDL levels in combination with high TG levels.

The incidence of CHD increases after menopause. National Cholesterol Education Program Adult Treatment Panel II (NHANES) has shown that total and LDL-cholesterol is accelerated in women after menopause [22]. Several clinical trials have shown that reducing LDL concentrations lowers the morbidity and mortality from coronary heart disease [22]. The most powerful agent that can reduce LDL levels is estrogen [23–25]. In the present study, a reduction of LDL was found in both treatment groups, but the change did not reach statistical significance in the CE/MPA group.

The changes in lipoproteins due to HRT are discussed in a review article [26]. It has been shown earlier that unopposed estrogen reduces LDL and increases HDL and TG levels in women with normal lipid levels. The reduction of LDL was confirmed in the present study, although only in the E2/NETA group. Compared to previous studies on cyclical HRT, continuous combined treatment was equally efficient in this respect [21]. Estrogens are known to reduce the synthesis of Lp(a) [27]. Independently of lipid changes, Lp(a) is regarded as a strong predictor for cardiovascular disease [3,28]. It has also been shown that the concentrations of Lp(a) are not influenced by external factors, such as exercise and dietary habits [29]. However, data from previous studies on effects of HRT on Lp(a) levels are inconclusive. Stadberg et al. [30] reported no significant effect on Lp(a) with 2 mg E2 and 1 mg NETA [30]. Others have shown that concomitant administration of MPA does not adversely affect an estrogen-induced reduction of Lp(a) concentrations in post-menopausal women [7]. We have shown that both treatment regimens reduce the Lp(a) concentrations.

There are limitations to the present study. When planning the study, 25 women in each treatment group was regarded as sufficient since that number is usually enough for studies on lipid effects. However, a post hoc power analysis on the found differences and standard deviations of differences in lipids between groups revealed that power was too low (<80%) concerning LDL and Lp(a). Thus, true differences between treatment effects that could have been seen in larger samples, might have been missed. Nevertheless, a significant reduction in LDL levels was seen in the E2/NETA group, and a significant reduction in Lp(a) levels in both groups. Another weakness is the drop out frequency, which was larger than expected, in the present study. However, the reasons for dropout (mainly bleeding problems) and the fact that there were no significant differences in baseline lipid values between completers and dropouts makes it hard to believe that the dropouts should have altered the results found.

These two continuous combined HRT's contain different types of estrogens (0.625 mg of CE and 2 mg micronized E2 respectively) but these doses are regarded as equipotent in their biological activity, at least in the endometrium [2,31]. Furthermore, similar effects of CE and E2 on plasma lipoproteins have been demonstrated earlier [32].

In addition to different estrogens, the two combinations used in our study also contained two different progestogens, namely MPA and NETA. To our knowledge, comparative studies of the effects on lipids and lipoproteins by 5 mg of MPA and 1 mg of NETA have not been performed before. It is not known if these two doses are equipotent in this respect. Earlier, high levels of TG in women treated with CE/MPA have been reported [7]. However, the TG increase was smallest in the group treated with continuous CE 0.625 mg/MPA 5 mg compared to lower continuous doses of MPA (2.5 mg), sequential MPA (5 and 10 mg) and CE regimen alone [7]. Estrogen alone tends to increase the concentrations of TG while progestogens, especially derivates of 19-nortestosterone, a more androgenic progestin, have been reported to reduce the TG levels [33]. In the three large randomized studies, WHI, HERS and PEPI, 2.5 mg MPA and 0.625 mg daily were administered [17,18,24], a combination frequently used in North America. The reason to use 5 mg MPA in the present study was and still is that, 2.5 mg MPA in combination with CE is not registered in Sweden. These clinical trials (WHI, HERS and PEPI) showed a decrease in total cholesterol and LDL, and an increase TG and HDL after 1 year of treatment. The increase in HDL levels during CE/MPA treatment could not be confirmed in the present study. A feasible explanation might be that the MPA dose used by us was higher. However, when combinations with 2.5 and 5 mg MPA were directly compared, no difference in HDL concentrations was noted [7].

Apart from dose differences, other factors might influence effects of HRT on lipids and lipoproteins. A recently published study comparing effects of HRT on lipids and lipoproteins in five European countries has reported national differences in cholesterol, HDL and Lp(a). A majority of the women in the study were treated with transdermal E2/NETA [34]. Conclusions from studies of lipids and lipoprotein metabolism during HRT cannot therefore be generalized to all populations.

There is evidence that HRT could affect thyroid function in post-menopausal women, and impaired thyroid function is known to affect lipid and lipoprotein profiles in a negative respect [35]. In the present study, treatment with E2/NETA reduced levels of HDL and possibly the elevation of TSH in this group could have contributed to this effect. However, this is less likely since both LDL and Lp(a) were reduced by E2/NETA treatment. Also, the levels of TSH were well below the normal range.

Due to the complexity of the interrelationships between the different lipid factors it is extremely difficult to interpret changes in lipid and lipoprotein concentrations during HRT and relate them to other risk factors for cardiovascular disease. The multiple regression analysis in the present study revealed no association between baseline body mass index or blood pressure and changes in lipoprotein levels. It has been suggested, that the addition of a progestogen to the estrogen treatment does not counteract the beneficial effects on lipoproteins in post-menopausal women [3,7]. However, the clinical relevance of this has been questioned after a recently published clinical trial [17]. The results from the present study show some differences between the two treatment groups. After 1 year of HRT significant differences between the two treatment groups regarding TC, HDL and TG were found. The CE/MPA group showed

intermediate changes in lipid profiles and the E2/NETA group exhibited greater changes.

At first site, the E2/NETA combination seems to be more beneficial regarding effects on lipid profile. Especially women with elevated lipid or lipoprotein levels before treatment might benefit more from this combination compared to the CE/MPA treatment. However, the single most important effect is probably the reduction of Lp(a), which was seen in both treatment groups. A finding that further complicates the interpretation of these data was the reduction of HDL seen in the E2/NETA group exclusively. Obviously, further research is needed concerning the effects on lipids of HRT, and (above all) clinical studies comparing effects on CHD with different preparations.

We conclude, that for the time being, none of the HRT combinations compared in this study should be recommended above the other (to women in general). In some cases though, an individualized choice of combination might be preferred based on the results of the study.

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EXHIBIT 4

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The beneficial effects of lipid-lowering drugs beyond lipid-lowering effects: A comparative study with pravastatin, atorvastatin, and fenofibrate in patients with type IIa and type IIb hyperlipidemia

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Abstract

Hyperlipidemia is an important risk factor for atherosclerosis. Hemorheological factors contribute to morbidity and mortality in patients with dyslipidemia. We evaluated the effects of 3 antihyperlipidemic drugs (pravastatin, atorvastatin, and fenofibrate), which have different mechanisms of action and different patterns of action on lipid profiles, on erythrocyte deformability and fibrinogen levels in patients with type IIa and type IIb hyperlipidemia. Twenty-one patients (4 men and 17 women) with type IIa and IIb hyperlipidemia were randomized to 3 drugs (pravastatin 20 mg/d, atorvastatin 10 mg/d, fenofibrate 250 mg/d) for 8 weeks. Plasma glucose, total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) analysis were performed on a BM-Hitachi 747-200 autoanalyzer (Hitachi-Roche, Tokyo, Japan). Fibrinogen analysis was performed according to Clauss method. Erythrocyte deformability was assessed with cell transit analysis device.There was no significant difference in body mass index, lipid profile, fibrinogen level, and erythrocyte deformability index values among the groups before treatment ($P > .05$). In all groups, there were statistically significant reductions in total LDL-C levels ($P < .05$). The triglyceride levels were significantly reduced in the atorvastatin and fenofibrate groups ($P < .05$), but not in the pravastatin group ($P > .05$). There was no significant change in HDL-C levels during the treatment with statins ($P > .05$), but there was a significant increase in the fenofibrate group ($P < .05$). Mean erythrocyte deformability index was improved in all the groups ($P < .05$). There was no significant change in fibrinogen levels during the treatment of pravastatin and atorvastatin ($P > .05$), but in fenofibrate group, fibrinogen levels were significantly decreased ($P < .05$).

The 3 groups of antihyperlipidemic drugs have beneficial effects on the erythrocyte deformability index. Only fenofibrate has significant beneficial effects on the fibrinogen levels.

1. Introduction

Hyperlipidemia is an important risk factor for atherosclerosis [1]. Epidemiological and clinical studies indicate that hyperlipidemia is associated with alterations in hemostatic and hemorheological factors [2,3]. Hemorheological factors such as blood viscosity, platelet activation state, and erythrocyte deformability contribute to morbidity and mortality in patients with dyslipidemia [4-7]. Erythrocyte deformation is an important regulatory factor of microcirculation [8]. Erythrocyte deformability deteriorates in patients with hyperlipidemia [3,9]. There are some reports that antihyperlipidemic treatment may improve erythrocyte deformability [9-13]. Although the effects of antihyperlipidemic drugs are well known, there is no concensus about the effects of these drugs on hemorheological parameters [14-16]. In one study, pravastatin was reported to significantly decrease plasma fibrinogen levels and plasma viscosity, but did not significantly change whole blood rheology in patients with familial hypercholesterolemia [9].

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In another study, lovastatin treatment decreased plasma viscosity and red cell aggregation, but did not change plasma fibrinogen level significantly [2]. Furthermore, Beigel et al [17] reported improvement in red blood cell filterability with an increase in fibrinogen levels with lovastatin treatment. As with the previous statins, there is also no consensus about the effect of atorvastatin [13,18].

The fibrate group is one of the oldest nonstatin medications for hyperlipidemia. Fibrates are normolipidemic drugs that decrease triglyceride and increase HDL concentrations in human beings. Fibrates are efficient drugs for the treatment of specific atherogenic lipid disorders, such as combined hyperlipidemia and hypoalphalipoproteinemia [19].

In this study, we compared the effects of 3 antihyperlipidemic drugs (pravastatin, atorvastatin, and fenofibrate) that have different mechanisms of action and different patterns of action on lipid profiles, on erythrocyte deformability, and on fibrinogen levels in patients with type IIa and type IIb hyperlipidemia.

2. Materials and methods

2.1. Patients

Twenty-one patients (4 men and 17 women; mean age, 51.7 ± 9.1 years) with type IIa (isolated hypercholesterolemia, LDL-C >160 mg/dL and triglycerides <200 mg/dL) and type IIb (mixed hyperlipidemia, LDL-C >160 mg/dL and triglycerides >200 mg/dL) hyperlipidemia were recruited and randomized to 3 groups. In each group, there were 7 age- and sex-matched patients. Groups A, B, and C were treated by pravastatin 20 mg/d, atorvastatin 10 mg/d, and fenofibrate 250 mg/d for 8 weeks, respectively. None of the patients had diabetes, renal failure, or myocardial infarction. Patients with endocrine, liver, hepatic, thyroid, and renal disorders, body mass index (BMI) of less than 30 kg/m^2, and alcohol abuse were excluded. Drugs with a known or potential effect on lipid levels or hemorheological parameters (β-blockers, thiazids, corticosteroids, estrogens, and aspirin) were not allowed during the study period. Informed consent was obtained from each subject before the study.

2.2. Methods

Weight and height were measured in light clothing without shoes. Body mass index was calculated as weight divided by height squared (kilograms per meter squared). The blood analysis was performed between 8:00 and 9:00 AM after 12 hours of fasting.

To maximize uniformity of diets and lifestyle habits, patients had dietary stabilization 6 weeks before the drug administration after counseling by a dietitian on the National Cholesterol Education Program Step 1 diet.

Plasma glucose, total cholesterol, triglyceride, and HDL-C, aspartate aminotransferase, alanine aminotransferase creatinine kinase, and lactate dehydrogenase levels were measured using SIGMA enzymatic kits (Sigma Diagnostics, Taufkirchen, Germany) in a BM-Hitachi 747-200 autoanalyzer (Hitachi-Roche, Tokyo, Japan). Low-density lipoprotein cholesterol was calculated by the Friedwald equation, except in patients with triglyceride levels higher than 400 mg/dL. Low-density lipoprotein cholesterol levels in patients with triglyceride levels higher than 400 mg/dL were measured using a bioMérieux Diagnostic (Lyon, France) LDL-C kit in a BM-Hitachi 747-200 autoanalyzer.

Fibrinogen analysis was performed according to the Clauss method [20].

Venous blood samples anticoagulated with heparin were analyzed to assess whole erythrocyte deformability within 30 minutes of sampling. Erythrocyte deformability was assessed by a cell transit analysis device. Erythrocyte deformability was assayed by measuring the erythrocyte filtration rate at which a 20% suspension of washed erythrocytes passed through nucleopore polycarbonate membrane with a 15-mm diameter and a pore of 5-μm filter [21-23].

Statistical tests were performed using a commercial software package (SPSS 8.0). Nonparametric tests (Mann-Whitney U test and Wilcoxon signed rank test) were used.

3. Results

3.1. Baseline parameters

In each group, there were sex- and age-matched 7 patients. As shown in the Table 1, there was no significant

Table 1
Baseline and posttreatment levels of lipid and hemorheological parameters

	Pravastatin (mean ± SE)		Atorvastatin (mean ± SE)		Fenofibrate (mean ± SE)	
	Pretreatment	Posttreatment	Pretreatment	Posttreatment	Pretreatment	Posttreatment
N	7	7	7	7	7	7
Male/female	1/6	1/6	2/5	2/5	1/6	1/6
BMI (kg/m^2)	24.8 ± 2.2	23.9 ± 2.3	25.9 ± 1.5	25.1 ± 1.4	25.6 ± 1.9	25.1 ± 2.0
Total cholesterol (mg/dL)	271 ± 45*	262 ± 26*	262 ± 26*	187 ± 32*	251 ± 37*	225 ± 45*
LDL-C (mg/dL)	186 ± 36*	141 ± 35*	174 ± 10*	104 ± 20*	172 ± 32*	140 ± 35*
HDL-C (mg/dL)	59 ± 15	61 ± 12	51 ± 3	56 ± 9	44 ± 14*	60 ± 16*
Triglyceride (mg/dL)	144 ± 77	109 ± 50	189 ± 94*	151 ± 78*	178 ± 95*	123 ± 66*
Fibrinogen (g/dL)	3.65 ± 0.75	4.26 ± 0.74	3.38 ± 0.84	3.39 ± 0.51	3.31 ± 0.60*	3.19 ± 0.62*
Erythrocyte deformability (ms/cell)	3.07 ± 0.16*	2.70 ± 0.26*	2.93 ± 0.22*	2.58 ± 0.12*	3.02 ± 0.15*	2.62 ± 0.14*

* $P < .05$ (pretreatment vs posttreatment).

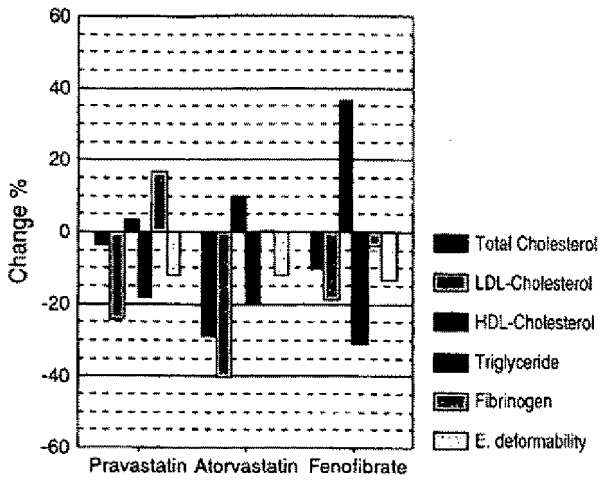

Fig. 1. Change in lipid and hemorheological parameters in all groups during the 8-week treatment period.

difference in BMI, lipid profile, fibrinogen level, and erythrocyte deformability index values among these 3 groups before treatment ($P > .05$).

3.2. Change in lipid parameters

As shown in the Table 1 and Fig. 1, in all groups, there were statistically significant reductions in total cholesterol and LDL-C levels ($P < .05$). The most prominent total cholesterol– and LDL-C–lowering effect (28.6%, 40.2%) was measured after atorvastatin treatment. The triglyceride levels were significantly reduced in atorvastatin (20.1%) and fenofibrate groups (31%) ($P < .05$), but not in pravastatin group (18.2%) ($P > .05$). There was no significant change in HDL-C levels during the treatment with statins (pravastatin [+3.38%] and atorvastatin [+9.8%]; $P > .05$), but there was a significant increase in fenofibrate group (+36.4%) ($P < .05$).

3.3. Change in hemorheological parameters

As shown in Fig. 1, the erythrocyte deformability index was improved in all groups in the posttreatment period ($P < .05$). There was no significant difference in the degree of erythrocyte deformability index improvement among these 3 groups ($P > .05$).

There was no significant change in fibrinogen levels during the treatment of atorvastatin and pravastatin group patients ($P > .05$). However, in fenofibrate group, fibrinogen levels were reduced significantly ($P < .05$).

4. Discussion

Our study revealed that hyperlipidemia treatment improves not only the lipid profile but also the hemorheological parameters.

In agreement with previous studies, patients receiving statin drugs had decreased total cholesterol and LDL-C levels [24]. The most prominent cholesterol-lowering effect was among patients administered atorvastatin [25]. Atorvastatin is a new generation 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) inhibitor of which metabolites stay longer in plasma and inhibit HMG-CoA reductase longer than the other statins [26].

Although pravastatin had no significant triglyceride-lowering effect, atorvastatin and fenofibrate had significant triglyceride-lowering effect. Atorvastatin has been shown to inhibit very low density lipoprotein (VLDL) secretion by limiting the availability of free cholesterol or cholesteryl esters for lipoprotein assembly, and it stimulates LDL receptors so the catabolism of VLDL and intermediate density lipoprotein increases. By these mechanisms, HMG-CoA reductase inhibitors lower triglyceride levels [25,26]. Fenofibrate administration significantly lowers both cholesterol and triglyceride levels. However, the most prominent effects with fenofibrate treatment were on triglyceride-lowering and HDL-increasing effects −31% and +36.4%, respectively. It is estimated that both enhanced catabolism of triglyceride-rich particles and reduced secretion of VLDL underlie the hypotriglyceridemic effect of fibrates [27-29]. Fibrates activate specific transcriptional factors belonging to the nuclear receptor superfamily, termed peroxisome proliferator-activated receptors (PPARs) [30]. The PPAR-α form mediates fibrate action on HDL-C levels via transcriptional induction of synthesis of the major HDL apolipoproteins, apoA-I and apoA-II. Previous reports revealed that fibrates increase HDL levels by approximately 15% to 25% [19,28,30]. However, in our study, we found 36.4% increase in HDL levels. These differences may result from the use of a limited number of fenofibrate-treated patients in our study. This finding has to be repeated in studies with greater numbers of patients. Although statins decrease all subtypes of LDL, fibrates mainly do not affect total LDL levels; they rather induce a shift in the LDL subtype distribution [31]. With these mechanisms, fibrates have beneficial effect on coronary artery disease [31].

In all groups, erythrocyte deformability was increased after administration of antihyperlipidemic drugs. In previous preliminary reports, erythrocyte deformability has been shown to increase not only with decreases in plasma cholesterol levels, but also with a decrease in plasma triglyceride levels [10,32-35]. Decrease in plasma cholesterol level was considered to be involved in the improvement of erythrocyte rheological properties by changing erythrocyte membrane phospholipid composition and phosphatidylcholine concentration [10,33,36,37]. Moreover, high plasma triglycerides could modulate the entry of cholesterol into platelets and thus decrease the free cholesterol-phospholipids ratio. The erythrocyte activities of various Na^+ or K^+ transport systems, including the Na^+-K^+ adenosine triphosphatase, the Na^+ leak, the Na^+-K^+ cotransport, and the Na^+-Li^+ countertransport, have also been shown to change with the plasma triglycerides level [12,33,38]. In our study, we could not find any significant difference in improvement of erythrocyte deformability index among patients treated with pravastatin,

atorvastatin, and fenofibrate. However, fenofibrate has an additional beneficial effect on fibrinogen levels that other agents do not have. Serum fibrinogen is directly correlated with plasma viscosity [6], which is also a cardiovascular risk factor [39]. As previously mentioned, fibrates exert their major effects via PPAR-α. Recently, it has been shown that PPAR-α regulates fibrinogen gene expression in rodents [30]. This animal study supports the results of our study. In addition, several previous studies also have shown that fenofibrate decreases fibrinogen in human beings [31,40-42]. Previous studies showed that there was no concensus about the effect of the statin on the fibrinogen levels [15,16]. The difference in results may be related to methodological factors. The immunologic assay is sensitive to fibrinogen as well as to fibrinogen degradation products, and thus may measure a higher value under certain conditions. In our study, the Clauss method that is not affected with the fibrinogen degradation products was used for fibrinogen levels.

5. Conclusion

These data show that these 3 groups of antihyperlipidemic drugs have beneficial effects on erythrocyte deformability index beyond their lipid-lowering effects. In addition, fenofibrate has significant beneficial effect on fibrinogen levels, which is one of the important cardiovascular risk factor.

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POSITION STATEMENT

EXHIBIT 5

Management of Dyslipidemia in Adults With Diabetes

AMERICAN DIABETES ASSOCIATION

RATIONALE FOR TREATMENT OF DYSLIPIDEMIA — The rationale for the treatment of diabetic dyslipidemia is discussed in detail in the American Diabetes Association (ADA) technical review "Management of Dyslipidemia in Adults With Diabetes" (1). Type 2 diabetes is associated with a two- to fourfold excess risk of coronary heart disease (CHD). Although the degree of glycemia in diabetic patients is strongly related to the risk of microvascular complications (retinopathy and renal disease), the relation of glycemia to macrovascular disease in type 2 diabetes is more modest. The finding of increased cardiovascular risk factors before the onset of type 2 diabetes also suggests that aggressive screening for diabetes combined with improved glycemic control alone will not be likely to completely eliminate excess risk of CHD in type 2 diabetic patients. Clearly, a multifactorial approach to prevention of CHD in type 2 diabetes will be necessary.

PREVALENCE OF DYSLIPIDEMIA IN TYPE 2 DIABETES — The most common pattern of dyslipidemia in type 2 diabetic patients is elevated triglyceride levels and decreased HDL cholesterol levels. The concentration of LDL cholesterol in type 2 diabetic patients is usually not significantly different from nondiabetic individuals. Diabetic patients may have elevated levels of non-HDL cholesterol (LDL plus VLDL). However, type 2 diabetic patients typically have a preponderance of smaller, denser LDL particles, which possibly increases atherogenicity even if the absolute concentration of LDL cholesterol is not significantly increased. Lastly, as shown in the technical review (1), the median triglyceride level in type 2 diabetic patients is <200 mg/dl (2.30 mmol/l), and 85–95% of patients have triglyceride levels below 400 mg/dl (4.5 mmol/l).

As in nondiabetic individuals, lipid levels may be affected by factors unrelated to glycemia or insulin resistance, such as renal disease, hypothyroidism, and the frequent occurrence of genetically determined lipoprotein disorders (e.g., familial combined hyperlipidemia and familial hypertriglyceridemia). These genetic disorders may contribute to the severe hypertriglyceridemia seen in some patients with diabetes. Furthermore, use of alcohol and estrogen may also contribute to hypertriglyceridemia.

LIPOPROTEIN RISK FACTORS FOR CHD — Relatively few prospective studies of lipids and lipoproteins as predictors of CHD have been reported in type 2 diabetic subjects, and the results have been somewhat contradictory. In the large Multiple Risk Factor Intervention Trial (MRFIT), total cholesterol as well as cigarette smoking and blood pressure predicted the development of cardiovascular disease in diabetic and nondiabetic subjects, suggesting that risk factors may be predictive in both groups. In a Finnish study, increased triglyceride levels and decreased HDL cholesterol levels (but neither LDL nor non-HDL cholesterol) predicted CHD in well-characterized type 2 diabetes subjects. However, after adjustment for HDL cholesterol, neither total nor VLDL triglyceride predicted CHD. Baseline data from the United Kingdom Prospective Diabetes Study (UKPDS) showed that both decreased HDL and elevated LDL predicted CHD (3). In observational studies, HDL may be the most consistent predictor of CHD in type 2 diabetes subjects, followed by triglyceride and total cholesterol.

CLINICAL TRIALS OF LIPID LOWERING IN DIABETIC SUBJECTS — No clinical trial has been done on the effects of lipid-lowering agents on subsequent CHD specifically in diabetic subjects. However, a number of clinical trials have included small numbers of adult type 2 diabetic subjects. In the Scandinavian Simvastatin Survival Study (4S) trial, simvastatin (HMG CoA reductase inhibitor or "statin") significantly reduced CHD incidence and total mortality (borderline significantly) in diabetic subjects with high LDL cholesterol and with previous clinical CHD. In the Cholesterol and Recurrent Events (CARE) study, pravastatin reduced CHD incidence significantly in diabetic subjects with average LDL levels and with previous clinical CHD. In the Helsinki Heart Study, gemfibrozil (fibric acid derivative) was associated with a reduction in CHD in diabetic subjects without prior CHD (although this result was not statistically significant). In the Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial (VA-HIT), gemfibrozil was associated with a 24% decrease in cardiovascular events in diabetic subjects with prior cardiovascular disease (4).

MODIFICATION OF LIPOPROTEINS BY MEDICAL NUTRITION THERAPY AND PHYSICAL ACTIVITY — The ADA has made recommendations for both medical nutrition therapy (MNT) (5) and physical activity (6). Weight loss and increased physical activity will lead to de-

The recommendations in this paper are based on the evidence reviewed in the following publication: Management of dyslipidemia in adults with diabetes (Technical Review). *Diabetes Care* 21:160–178, 1998.

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Abbreviations: ADA, American Diabetes Association; CARE, Cholesterol and Recurrent Events Study; CHD, coronary heart disease; CVD, cerebrovascular disease; MNT, medical nutrition therapy; NCEP, National Cholesterol Education Program; 4S, Scandinavian Simvastatin Survival Study.

Table 1—*Category of risk based on lipoprotein levels in adults with diabetes*

Risk	LDL cholesterol	HDL cholesterol*	Triglyceride
High	≥130	<40	≥400
Borderline	100–129	40–59	150–399
Low	<100	≥60	<150

Data are given in milligrams per deciliter. *For women, the HDL cholesterol values should be increased by 10 mg/dl.

Table 2—*Treatment decisions based on LDL cholesterol level in adults with diabetes*

	Medical nutrition therapy		Drug therapy	
	Initiation level	LDL goal	Initiation level	LDL goal
With CHD, PVD, or CVD	≥100	<100	≥100	<100
Without CHD, PVD, and CVD	≥100	<100	≥130*	<100

Data are given in milligrams per deciliter. *For patients with LDL between 100 and 129 mg/dl, a variety of treatment strategies are available, including more aggressive MNT and pharmacological treatment with a statin; in addition, if the HDL is <40 mg/dl, a fibric acid such as fenofibrate may be used in these patients. MNT should be attempted before starting pharmacological therapy. PVD, peripheral vascular disease.

creased triglyceride and increased HDL cholesterol levels and also to modest lowering of LDL cholesterol levels. Diabetic patients who are overweight should be given a prescription for MNT and for increased physical activity. The proportion of saturated fat in the meal plan should be reduced. The ADA suggests an increase in either carbohydrate or monounsaturated fat to compensate for the reduction in saturated fat. Some (but not all) studies suggest that a high–monounsaturated fat diet may have better metabolic effects than a high-carbohydrate diet, although other experts have suggested that such a dietary modification may make weight loss more difficult in obese diabetic patients.

Recommendations of the American Heart Association for patients with CHD (7) have suggested that the maximal MNT typically reduces LDL cholesterol 15–25 mg/dl (0.40–0.65 mmol/l). Thus, if the LDL cholesterol exceeds the goal by >25 mg/dl (0.65 mmol/l), the physician may decide to institute pharmacological therapy at the same time as behavioral therapy for high-risk patients (i.e., diabetic patients with a prior myocardial infarction and/or other CHD risk factors). In other patients, behavioral interventions may be evaluated at 6-week intervals, with consideration of pharmacological therapy between 3 and 6 months.

MODIFICATION OF LIPOPROTEINS BY GLUCOSE-LOWERING AGENTS — Interventions to improve glycemia usually lower triglyceride levels. In general, glucose-lowering agents do not change or have only a modest effect on raising HDL levels. However, the HDL composition may change in a direction thought to be antiatherogenic. Thiazolidinediones may increase HDL and LDL levels, but the long-term effect of such changes is not known. LDL cholesterol may decrease modestly (up to 10–15%) with the achievement of optimal glycemic control. Since improved glycemic control may also lower triglyceride levels, it might also cause a favorable change in LDL composition.

TREATMENT GOALS FOR LIPOPROTEIN THERAPY — The categories of CHD risk by lipoprotein levels in type 2 diabetic patients are shown in Table 1. Because of frequent changes in glycemic control in diabetic patients and their effects on levels of lipoprotein, levels of LDL, HDL, total cholesterol, and triglyceride should be measured every year in adult patients. If values fall in lower-risk levels, assessment may be repeated every 2 years. In children with diabetes, consideration should be given to measuring lipoproteins after age 2 years, as suggested by the National Cholesterol Education Program (NCEP) Report of the Expert Panel on Blood Cholesterol in Children and Adolescents (8).

Optimal LDL cholesterol levels for adults with diabetes are <100 mg/dl (2.60 mmol/l), optimal HDL cholesterol levels are >40 mg/dl (1.02 mmol/l), and desirable triglyceride levels are <150 mg/dl (1.7 mmol/l). (In women who, at least when nondiabetic, tend to have higher HDL cholesterol levels than men, it may be desirable to have even higher HDL cholesterol levels [>50 mg/dl (1.28 mmol/l)].) However, raising HDL cholesterol levels pharmacologically in diabetic patients is very difficult since the most effective agent raising HDL cholesterol levels is nicotinic acid, which is relatively contraindicated in diabetic patients. Fibrates can raise HDL cholesterol levels significantly without affecting glycemic control.

The recommendations for treatment of elevated LDL cholesterol (Table 2) generally follow the guidelines of both the NCEP (9) and a recent ADA consensus development conference (1993) (10), with the following caveats. Pharmacological therapy should be initiated after behavioral interventions are used. However, in patients with clinical cardiovascular disease or very high LDL cholesterol levels (i.e., ≥200 mg/dl [5.15 mmol/l]), pharmacological therapy should be initiated at the same time that behavioral therapy is started.

In the context of the NCEP report, it is suggested that diabetic subjects with clinical CHD and an LDL cholesterol level of ≥100 mg/dl (2.60 mmol/l) after MNT and glucose interventions be treated with pharmacological agents. For diabetic patients without pre-existing CHD, the current ADA recommendations for starting pharmacological therapy are 1) an LDL cholesterol level of ≥130 mg/dl (3.35 mmol/l) and 2) a goal of <100 mg/dl (2.60 mmol/l) for LDL cholesterol. These recommendations are based not only on the high incidence of CHD in patients with diabetes, but also on their higher case fatality rate once they have CHD. Since a large proportion of diabetic patients die before they reach the hospital, a preventive strategy based solely on secondary prevention would not be able to "save" large numbers of these diabetic patients. In patients with LDL between 100 mg/dl (2.60 mmol/l) and 129 mg/dl (3.30 mmol/l), a variety of treatment strategies are available, including more aggressive MNT and pharmacological treatment with a statin (11). MNT should be attempted before starting pharmacological therapy. In addition, if the HDL is <40 mg/dl, a fibric acid such as fenofibrate might be used in patients with LDL between 100 and 129 mg/dl.

In agreement with the earlier ADA consensus panel (10), increased triglyceride levels are recognized as a target for intervention. Since recommended LDL levels are considered to be <100 mg/dl (2.60 mmol/l), and since many diabetic patients have increased triglyceride levels, a large proportion of diabetic patients will have elevated levels of both LDL cholesterol and triglycerides. As such, there is likely to be an increase of diabetic patients on pharmacological therapy and thus an increase in expendi-

Table 3—*Order of priorities for treatment of diabetic dyslipidemia in adults

I. LDL cholesterol lowering*
  First choice
    HMG CoA reductase inhibitor (statin)
  Second choice
    Bile acid binding resin (resin) or fenofibrate
II. HDL cholesterol raising
  Behavioral interventions such as weight loss, increased physical activity, and smoking cessation may be useful
  Difficult except with nicotinic acid, which should be used with caution, or fibrates
III. Triglyceride lowering
  Glycemic control first priority
  Fibric acid derivative (gemfibrozil, fenofibrate)
  Statins are moderately effective at high dose in hypertriglyceridemic subjects who also have high LDL cholesterol
IV. Combined hyperlipidemia
  First choice
    Improved glycemic control plus high-dose statin
  Second choice
    Improved glycemic control plus statin† plus fibric acid derivative† (gemfibrozil, fenofibrate)
  Third choice
    Improved glycemic control plus resin plus fibric acid derivative (gemfibrozil, fenofibrate)
    Improved glycemic control plus statin† plus nicotinic acid† (glycemic control must be monitored carefully)

*Decision for treatment of high LDL before elevated triglyceride is based on clinical trial data indicating safety as well as efficacy of the available agents. †The combination of statins with nicotinic acid and especially with gemfibrozil or fenofibrate may carry an increased risk of myositis. See text for recommendations for patients with triglyceride levels >400 mg/dl.

tures on pharmacological therapy. However, the clinical trial data suggest that reduction of LDL cholesterol is associated with reduction in CHD and perhaps overall mortality.

Economic analyses, based on the 4S study, suggest that pharmacological therapy may be cost-effective once indirect costs of CHD are taken into account (12).

Table 3 shows the order of priorities for treatment of dyslipidemia. Treatment of LDL cholesterol is considered as the first priority for pharmacological therapy of dyslipidemia for a number of reasons (1). Clinical trials (4S and CARE) showing the effectiveness of statins in reducing CHD in diabetic subjects show greater risk reductions with narrower confidence intervals than the Helsinki Study with gemfibrozil.

The initial therapy for hypertriglyceridemia is behavioral modification with weight loss, increased physical activity, and moderation of alcohol consumption. In the case of severe hypertriglyceridemia (≥1,000 mg/dl [11.3 mmol/l]), severe dietary fat restriction (<10% of calories) (in addition to pharmacological therapy) is necessary to reduce the risk of pancreatitis. Improved glycemic control (which has been facilitated by the introduction of new glucose-lowering agents and more frequent use of combination therapy) is also very effective for reducing triglyceride levels and should be aggressively used before the introduction of fibric acids. After the achievement of optimal glycemic control (or at least after the achievement of as much improvement as likely to be possible), the physician may consider adding a fibric acid. In Table 1, the decision to start pharmacological therapy treatment is dependent on the clinician's judgment between triglyceride levels of 200 mg/dl (2.30 mmol/l) and 400 mg/dl (4.50 mmol/l). Above 400 mg/dl (4.50 mmol/l), strong consideration should be given to pharmacological treatment of triglyceridemia. In contrast, improved glycemic control will only modestly reduce LDL cholesterol levels, and therefore in diabetic patients with both high LDL cholesterol and high glucose levels, one might simultaneously initiate glucose lowering and statin therapy. In some studies, higher-dose statins are moderately effective in reducing triglyceride levels in markedly hypertriglyceridemic subjects (triglyceride ≥300 mg/dl [3.40 mmol/l]). The critical issue is that gemfibrozil should not be initiated alone in diabetic patients who have undesirable levels of both triglyceride and LDL cholesterol. Fenofibrate, a recently approved fibric acid derivative, may have greater LDL-lowering effects and may be useful in diabetic patients with combined hyperlipidemia. Although HDL cholesterol, as noted above, is a powerful predictor of CHD in diabetic patients, it is difficult to raise HDL cholesterol levels without pharmacological intervention. Nicotinic acid, which should be used with caution in diabetic patients, and fibrates can effectively increase HDL cholesterol levels. Behavioral interventions (weight loss, smoking cessation, increased physical activity) may increase HDL cholesterol.

In some cases, combined lipid therapy may be initiated. Several options are shown in Table 3. The combination of statins with nicotinic acid and especially with gemfibrozil or fenofibrate has been associated with increased risk of myositis, although the risk of clinical myositis (as opposed to elevated creatinine phosphokinase levels) appears to be low. However, the risk of myositis may be increased with the combination of gemfibrozil and cerivastatin or in patients with renal disease. The combination of statins with nicotinic acid is extremely effective in modifying diabetic dyslipidemia (with the largest increases in HDL cholesterol levels), but the combination may significantly worsen hyperglycemia. Thus, this combination should be used with extreme caution: use low doses of nicotinic acid (≤2 g of nicotinic acid per day) with frequent monitoring of glucose levels.

LIPID-LOWERING AGENTS — A brief summary of the actions of available agents for lipid lowering in patients with diabetes is shown in Table 4. Generally, one or two agents are available in each class with the exception of the statins, for which there are many. The choice of statin should depend principally on the LDL reduction needed to achieve the target (<100 mg/dl [2.60 mmol/l]), on the initial LDL level, and on the judgment of the treating physician.

It should also be noted that the higher doses of statins may be moderately effective at reducing triglyceride levels (though not necessarily at raising HDL levels) and thus may reduce the need for combination therapy. With the use of high doses of statins, the LDL levels may be reduced to 80 mg/dl (2.05 mmol/l) or less, and there is no safety data at such low LDL levels. The use of very high-dose statin therapy (i.e., simvastatin 80 mg or atorvastatin 40 or 80 mg) to treat hypertriglyceridemia should be restricted to patients with both high levels of LDL cho-

Table 4—*Pharmacologic agents for treatment of dyslipidemia in adults*

	Effect on lipoprotein			Clinical trials in diabetic subjects
	LDL	HDL	Triglyceride	
First-line agents				
LDL lowering				
HMG CoA reductase inhibitor	↓↓	↔↑	↔↓	4S (simvastatin) CARE (pravastatin)
Triglyceride lowering				
Fibric acid derivative	↓↔↑	↑	↓↓	Helsinki (gemfibrozil)
Second-line agents				
LDL lowering				
Bile acid binding resins	↓	↔	↑	None
LDL and triglyceride lowering				
Nicotinic acid	↓	↑↑	↓↓	None

In diabetic patients, nicotinic acid should be restricted to ≤2 g/day; short-acting nicotinic acid is preferred.

lesterol as well as high triglyceride levels. Changes in therapy should be done at ~4- to 6-week intervals based on laboratory findings.

CONSIDERATIONS IN THE TREATMENT OF ADULTS WITH TYPE 1 DIABETES

— Type 1 diabetic patients who are in good control tend to have normal (and sometimes better than normal) levels of lipoprotein. Their composition of lipoproteins may be abnormal, but the effects of these compositional abnormalities in relation to CHD are unknown. There is relatively little observational data on lipoproteins and CHD, and there are no clinical trials relating lipoproteins to CHD. It seems reasonable that if type 1 diabetic patients have LDL cholesterol levels that are above the goals recommended for type 2 diabetic patients (Table 2), they should be aggressively treated. Improved glycemic control may be even more important in type 1 diabetic patients than in type 2 diabetic patients for reduction of CHD (e.g., Wisconsin Epidemiologic Study of Diabetic Retinopathy [WESDR]).

CONCLUSIONS

— Aggressive therapy of diabetic dyslipidemia will probably reduce the risk of CHD in patients with diabetes. Primary therapy should be directed first at lowering LDL levels. The goal is to reduce LDL concentrations to levels recommended for patients with pre-existing CHD (≤100 mg/dl [2.60 mmol/l]). The initiation level for behavioral interventions is also an LDL cholesterol of >100 mg/dl (2.60 mmol/l)]. The initial therapy should be to use statin therapy with the addition of a resin if necessary to reach the LDL goal.

However, limited data are available from clinical trials, especially in diabetic patients without clinical cardiovascular disease. In the absence of such data, because of the high mortality for diabetic patients with first myocardial infarction, aggressive treatment of dyslipidemia is also indicated. For patients without previous CHD, the goal for LDL cholesterol is ≤100 mg/dl (2.60 mmol/l); the initiation level for pharmacological therapy is set at an LDL level ≥130 mg/dl (3.35 mmol/l). However, for patients with LDL levels between 100 and 129 mg/dl, a variety of treatment strategies are available, including more aggressive MNT and pharmacological treatment with a statin. MNT should be attempted before starting pharmacological therapy. In addition, if the HDL is <40 mg/dl, a fibric acid such as fenofibrate might be used in patients with LDL cholesterol between 100 and 129 mg/dl.

The initial therapy for hypertriglyceridemia is improved glycemic control. Additional triglyceride lowering can be achieved with very high dose statins (for subjects with both high LDL and triglyceride levels) or fibric acid derivatives (gemfibrozil or fenofibrate).

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RELATED PROCEEDINGS APPENDIX

None